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Plant strategies in belowground competition – insight through game theory

Strategie rostlin v podzemní kompetici – náhled skrze teorii her

Diploma thesis

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I hereby declare that I completed this master thesis independently and that I have properly acknowledged and cited all used sources. No part of the thesis was used to obtain different or the same academic degree.

Prohlašuji, že jsem závěrečnou práci zpracoval samostatně a že jsem uvedl všechny použité informační zdroje a literaturu. Tato práce ani její podstatná část nebyla předložena k získání jiného nebo stejného akademického titulu.

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Note

Chapters 1.1, 2 and 5.1 are a part of manuscript that is currently submitted under name **"Doves-and-Hawks belowground: Root competition strategies and phylogeny"** to Journal of Ecology. Author contributions to the manuscript are: **Jan Smyčka**: formulation of idea, modelling, meta-analysis, writing; **Tomáš Herben**: critical discussion of both idea and text, key advises with meta-analysis.

Summary

In recent decades, it was shown that belowground competition for some plants may take form of the tragedy of the commons (TOC). In these plants, the competing neighbours invest more in root systems than would be appropriate for optimal nutrient uptake for the group and also more than they do when grown alone. However, there is also strong evidence that other species do not follow TOC, and tailor their root system to best nutrient exploitation irrespectively of competitor presence. The root investment strategy of these plants should correspond to the ideal free distribution (IFD).

In my thesis I focus on two aspects:

- I use game theoretical models to explore, whether those strategies can coexist within species and also whether different species can coexist if they have different strategy. From this model I draw predictions, which I test by meta-analysis.
- Using *Agrostis stolonifera* as a model, I test assumptions on nutrient and neighbour perception, which underlie TOC and IFD models.

I show that according to mathematical models, those two strategies can coexist in different species in a community, but cannot coexist within a species. Within a species, the TOC strategy should always dominate, once it appears. This can be extrapolated to macroevolutionary scale – once TOC occurs in certain clade, it should not disappear. By meta-analysis of strategies across different species, I show that this really is the case, with TOC strategy clustered to Fabaceae or rosids clade.

A. stolonifera strongly decreases investment to roots in presence of competitor. This is something that does not match either TOC or IFD strategy. This species also avoids competitors' root system in space. By analysis of rhizosphere shape, I show that this behaviour is strongly governed by competitor presence, rather by nutrient availability.

It is possible to conclude, that root overproduction in intraspecific competition is likely evolutionary novelty of legumes or the rosids clade. However, due to anomalous ecology of Fabaceae, it is not clear whether this overproduction really is expression of TOC. In addition, it is clear that there are species that do follow neither TOC nor IFD. Those two findings together make the whole idea of rooting strategies dichotomisation to TOC and IFD questionable.

Abstrakt

V posledních desetiletích se ukazuje, že některé rostliny po vystavení kořenové kompetici zažívají tragedii obecních pastvin (TOC). V přítomnosti kompetitora investují do podzemní biomasy více, než by odpovídalo optimální výtěžnosti živin celé kompetující skupiny, a také více než když kompetici vystaveny nejsou. Nicméně se ukazuje, že jiné druhy se do TOC nezapojují a pravděpodobně svůj kořenový systém přizpůsobují pro nejlepší výtěžnost živin, nezávisle na přítomnosti kompetitora. Investice těchto rostlin do kořenů se pravděpodobně řídí ideální volnou distribucí (IFD).

Ve své diplomové práci se zaměřuji především na dva aspekty:

- Pomocí matematického modelování zkoumám, jestli tyto dvě strategie mohou koexistovat v rámci jednoho druhu, případně jestli druhy s různými strategiemi mohou existovat v jednom společenstvu. Z výsledků modelování odvozuji predikce, které následně testuji meta-analytickou studií.
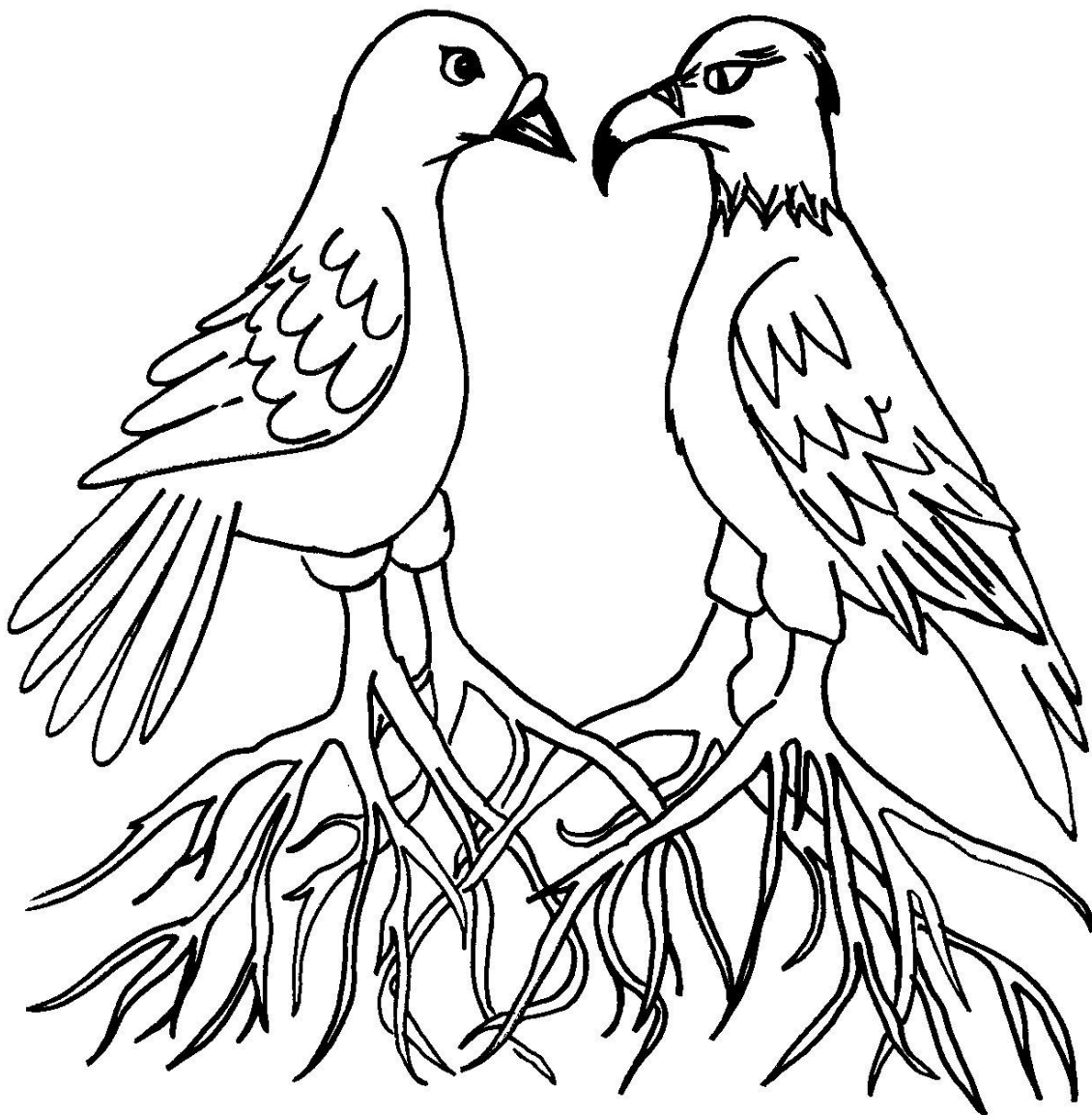- Na druhu *Agrostis stolonifera* testuji předpoklady TOC a IFD modelů ohledně vnímání sousedů a živin.

Pomocí matematického modelu ukazuji, že tyto dvě strategie pravděpodobně mohou koexistovat v rámci společenstva, nicméně koexistence v rámci druhu není možná. Uvnitř druhu by měla vždy převládnout strategie vedoucí k TOC. Z toho je možné odvodit, že jakmile nějaká evoluční větev získá TOC strategii, je velmi nepravděpodobné, aby došlo k jejímu přepnutí na IFD. Pomocí metaanalýzy kompetičních strategií napříč různými druhy ukazuji, že to tak opravdu je, a strategie vedoucí k TOC je typická pro čeleď Fabaceae, nebo širší okolí této čeledi (tzv. rosids clade).

A. stolonifera výrazně snižuje investici do kořenového systému v přítomnosti kompetitora. To je výsledek, který neodpovídá TOC ani IFD modelu. Tento druh se navíc v prostoru výhýbá kořenům kompetitora. Pomocí analýz tvaru kořenového systému ukazuji, že toto chování je daleko více závislé na vnímání přítomnosti kompetitora, než na vnímání dostupnosti živin.

Na základě těchto výsledků je možné shrnout, že nadprodukce kořenů ve vnitrodruhové kompetici je pravděpodobně evoluční novinkou bobovitých nebo jejich širšího okolí. Nicméně, vzhledem k specifické ekologii této čeledi není zřejmé, jestli je tato nadprodukce skutečně projevem TOC. Navíc je zřejmé, že existují druhy, které nezastávají strategii vedoucí k TOC ani IFD. Tato dvě zjištění naznačují, že celý koncept popisující podzemní kompetici pomocí TOC nebo IFD , nestojí na příliš pevných základech.

Acknowledgement

Content

1. Introduction

Plants' behaviour in root competition, although often overlooked due to technical difficulties associated with its study, is very important for shaping communities and populations (Schenk 2006). Root competition may appear seemingly less important than aboveground competition, because relations between individuals belowground are more symmetric than aboveground (Weiner 1990). Weaker individuals in belowground competition get amount of resources proportional to their weight, compared to aboveground competition, where few strongest individuals receive all the benefit. However, symmetry in belowground competition takes place on scales of individual lifespan. Over evolutionary time scale, "systematic losers" in belowground competition are likely to be excluded as well as individuals, which do not perform well aboveground. Thus, decisions about investment to roots, spatial placement of roots, or reaction to competitor are important ecological and evolutionary traits. In competitive situations, fitness of one plant individual is not dependent only on its own decisions, but also on decisions of competitors. If this is true, optimal plants' decisions become strategies – complex battle-plans that consider also behaviour of opponent. Here we leave the grey world of trait optimisations and enter the wonderland of evolutionary game theory.

1.1. Tragedy of commons of root investment in competition

Several papers in the last decade have shown that many plant species follow the "tragedy of the commons" rooting strategy (TOC) when they face belowground intraspecific competition (Gersani et al. 2001; Maina et al. 2002; O'Brien et al. 2005). Individuals with this type of behaviour invest disproportionately more resources to belowground biomass than would maximize total nutrient uptake efficiency for all competing individuals together. For example, O'Brien et al. (2005) showed that peas plants grown as pairs in pots of volume V have increased root/shoot ratios and decreased reproductive performances compared to plants grown separately in pots of volume $V/2$. Using simple cost-benefit model with implicit time and space, it was also shown that this strategy is evolutionarily stable, i.e. once it prevails in population, it cannot be invaded by any other strategy (Gersani et al. 2001; O'Brien and Brown 2008; McNickle and Brown 2012; McNickle and Dybzinski 2013).

Surprisingly, there is also strong evidence for plant species that not use the TOC strategy (Semchenko et al. 2007a; Nord et al. 2011; McNickle and Brown 2014). In competition, these plants seemingly maximize "common good" at the expense of individual success. In their recent work, McNickle and Brown (2014) propose an explanation for this strategy. Using a cost-benefit model similar to Gersani et al. (2001), they suggest an optimal rooting strategy for plants that do not distinguish between competitive and non-competitive situations (game-off, in

contrast to game-on plants involved in the TOC strategy). These plants thus tailor their rooting strategy only to nutrient availability regardless of competitor presence. This modification leads to a rooting strategy that follows the ideal free distribution (IFD, sensu Fretwell and Lucas 1968) and does not exhibit root overproduction in competition, which is typical for TOC.

However, the ecological and evolutionary drivers determining which strategies are pursued by species have yet to be identified. Also, it is not clear whether one species must be uniformly game-off or game-on, or whether there can be intraspecific variation, and thus coexistence between strategies within one species can occur. The evolutionary stability of the game-on strategy suggests that it should dominate and exclude the game-off strategy in most situations. Extrapolated to the macroevolutionary scale, this would imply great phylogenetic conservatism of the game-on strategy, i.e. once the game-on strategy appears in a clade, it would be extremely improbable to switch to game-off.. McNickle and Brown (2014), on the other hand, point out that the coexistence of both strategies may be enabled by the fact that nutrient uptake is not the ultimate value for plants and that it must be translated to fitness, which is also shaped by other processes, such as aboveground competition, reproductive strategy and other biotic interactions. The assignment of the game-on strategy to species and populations would thus be driven by plants' actual ecological context rather than evolutionary history.

Interestingly enough, most of the literature on rooting strategy in competition does not clearly distinguish between interspecific and intraspecific competition strategy, and often mix these phenomena together (see Semchenko et al. 2007; O'Brien and Brown 2008; McNickle and Brown 2014). This is surprising, because there is quite strong evidence that one plant species can behave differently under intraspecific and interspecific competition (Semchenko et al. 2007b; Haase 2009; Poorter et al. 2012; Mommer et al. 2012; Padilla et al. 2013). For example Haase 2009 shows that TOC in seedlings of trees is stronger in interspecific competition than in the intraspecific one. However, interspecific competition probably is not stronger in general: *Glechoma hederacea* shows strong avoidance patterns to presence of *Fragaria vesca*, having neutral reaction to conspecifics at the same time (Semchenko et al. 2007b). In contrast, *F. vesca* in the same experiment exhibits more aggressive behaviour towards *G. hederacea*, than to the conspecifics. Generalisation of patterns in interspecific competition behaviour is also complicated by the fact, that reaction of the focal species can be also different towards different competitor species (Semchenko et al. 2013). Nevertheless, it is possible to conclude that rooting behaviour may differ in inter and intraspecific competition. Thus it may be interesting to explore evolutionary forces which differ in inter- and intraspecific competition.

Competitor identity may influence competitive behaviour even on scales lower than species. For example plants of *Arabidopsis thaliana* were shown to have different rooting patterns when they

are grown with sibling or with different genotype (Biedrzycki et al. 2010). Study across many species has revealed that phenomena of kin recognition in competition is not very common in temperate grasslands (Lepik et al. 2012). This is quite surprising, because according to Hamilton rule (Hamilton 1964), plants should have strong motivation to coordinate with their kin. However, evidence for kin recognition seems to be quite consistent for one special case – kin recognition of different ramets within the clone. This has been demonstrated for many clonal species with contrasting ecology and phylogenetic position (*Buchloe dactyloides*, Gruntman and Novoplansky 2004; *Fragaria chiloensis*, Holzapfel and Alpert 2003; *Fragaria vesca*, *Glechoma hederacea*, Semchenko et al. 2007b). There are three explanations, why kin recognition is more common within a clone than within sexual kin. One is that plants within a clone have stronger motivation to recognise themselves and cooperate. This is because they are really 100% relative, compared to sexuals, where the relatedness is 50% or less (Hamilton 1964). Another one is that clonal offspring usually are not dispersed very far, which means that it is more probable for clonal species that they will meet the kin (Doust 1981). However, length of clonal spread is object of evolution as well, and may be optimized to minimize competition costs (Herben and Novoplansky 2007; Weiser and Smyčka 2015). Third explanation is, that persisting connection between two ramets enables some kind of signalisation that is necessary for kin recognition. This is in accord with the evidence from *Buchloe dactyloides*: ramets behave more cooperatively to others ramets, when they still have active connection to them, compared to the situations when connection was cut (Gruntman and Novoplansky 2004; Holzapfel and Alpert 2003).

1.2. Root navigation and signalisation in competition

Importance of information perception in root competition is remarkable on distinction between TOC and IFD rooting strategy. Fundamental difference between game-on and game-off plant is, that game-on plant simply takes into an account more information about its competitive situation. Game-on plant "knows" that nutrients decrement is caused by neighbour and thus will be dependent on neighbours' prosperity in future (McNickle and Brown 2014). For understanding root competition strategies in whole complexity, it might be better to track what kinds of information plant can obtain about its belowground situation (as in Novoplansky 2009), rather than focusing on specific phenomena, as is root overproduction in competition. This is, however, rather problematic from the practical point of view, because many of phenomena of "plants behaviour" were described only for single species, which hardly enables any generalisations. Thus we lack broader evidence that would allow extrapolations of behavioural patterns to unexplored plant species. This is probably because ecological plant sciences lack real model organisms, to which more knowledge would be aggregated. Of course, well described is

information perception in species like *Arabidopsis thaliana* or several field crops. But all those models are annuals from inbred lineages where artificial selection takes place, and thus very specific from both ecological and evolutionary point of view. I'll show few aspects of plant information perception and orientation belowground, which are either considered general across many plant species or which may be crucial for behaviour in competition.

Main purpose of having roots for most plant species is getting the nutrients and water from soil (Fitter 1999). This means that nutrient concentration should be very important information for making decisions about root investment and spatial placement. There is a lot of evidence that plants can perceive nutrient concentrations and tailor root system to it (Drew 1975; Neumann and Römheld 1999; Caffaro et al. 2013). Also most of theories and formal models on plant rooting in competition lie on assumption, that plants tailor their behaviour to perceived nutrient concentrations in soil (Gersani et al. 2001; Craine 2006; Hess and De Kroon 2007; Novoplansky 2009; McNickle and Brown 2014). However, implementation of nutrients perception and economy in those models is usually over-simplified in three ways:

(i) Some of those models, and also less formal ideas developed upon those, are spatially implicit (Gersani et al. 2001; Hess and De Kroon 2007; Semchenko et al. 2007a; McNickle and Brown 2014), and ignore that purpose of roots in not only foraging for nutrients, but also transport to shoot. This transportation of course incurs additional costs to resource exploitation process that are higher with increasing distance from the shoot. It means, that in rooting space shared by competitors, there are patches that are more advantageous for one of competitors, just because they are closer. Some other approaches take those transportation costs in account (Novoplansky and Cohen 1997; Craine et al. 2005; O'Brien et al. 2007). Although those spatially explicit models take in account increased costs of foraging in distant patches, they omit that distant foraging also positively affects amount of root biomass, because transportation structures in plant body can have considerable mass (West et al. 1999). In common experiments, it is usually impossible to separate one foraging and transportation biomass, and especially in case of nutrient poor or patchy environments, it should be taken in account that considerable part of root biomass may not serve for foraging, but rather for transportation.

(ii) In heterogeneous environments, also searching for nutrients might have an important effect on root mass investment and spatial pattern (reviewed in Hodge 2004). It is easy to imagine a situation, where plant invests to exploration towards poor soil space, with expectation of finding better patches further in that direction (similar effect is shown in Cahill et al. 2010). In other words, the expected trend might be more important for root behaviour than actual nutrient concentrations, and plant thus has to integrate "expectations" to its rooting behaviour. This is effect that has also been shown for temporal heterogeneity. For example, it has been shown, that

pea plants allocate roots more to relatively poor patches with increasing amount of nutrients, than to patches with stable high nutrient levels (Shemesh et al. 2010; Shemesh and Rosen 2011).

(iii) Typically it is also assumed that plants aim on higher gain of nutrients. This is of course true, if plant is limited by nutrient availability. However, in case when plant is limited by light, water or for example availability of pollinators, it should, according to Liebig law of minimum, focus rather on getting other resources than soil nutrients. Plants thus have to integrate information about nutrients, water availability, aboveground limitations and other resources, and focus on obtaining the most limited resource (as in Dybzinski et al. 2011). This can be for example demonstrated in experiments with plants facing to different intensities of aboveground or belowground competition. Here, ones limited by aboveground resources invest more to shoot biomass, and those limited by soil resources to root biomass (Weiner 1986; Wang et al. 2014; Kiaer et al. 2013). Even more problematic for description by the competition models is the situation, where plants have too much of soil resources. Plant fitness can be significantly lowered by water or nutrients oversupply (Chapin 1980; Silvertown et al. 1999). In this case, positive navigation of roots by nutrient concentrations does not make much sense. From the point of view of competitive behaviour, over-fertilisation could theoretically lead even to facilitation, where competitors mutually lower the concentration of toxic nutrient to each other.

Although nutrient concentration is probably most universal type of information, which plants use for root navigation, there are also others. One of those discussed in context of competition is detection of physical obstacles. There is an evidence from classical literature showing, that plants can react to physical obstacle (such as large soil particle), when they get to physical contact with it (Darwin and Darwin 1880 pp 129; Fitter 1999 pp 719; Kozlowski 1999; Clark et al. 2003). As well, plants can detect competitor roots by physical contact (Mahall and Callaway 1992). Lately, it has been also shown, that pea plants can detect solid object also from distance. Falik et al. (2005) show, that pea rooting behaviour can be altered by presence of nylon string in soil, that resembles root by shape, but is chemically inert. This can be explained by accumulation of chemical compounds produced by roots (exudates) near the impermeable physical obstacle. Root growth is then self-inhibited by those exudates. It means that plants can detect competitor roots using mechanism, which does not bring any additional information on species identity or relatedness of competitor. Such a finding can be especially important for exploring relations between behaviour in interspeficic and intraspecific root competition.

A special case of obstacle detection, where root system is completely surrounded by solid obstacle, is plants ability to tailor rooting to the size of the pot. There is a lot of evidence, that plants can do this (McConnaughay and Bazzaz 1991; NeSmith and Duval 1998; O'Brien et al. 2005; Chen et al. 2015). Usually plants in larger pot produce larger root systems. This fact was

used to criticise the design of experiments showing TOC described in previous chapter. The root over-proliferation in competition treatment, in comparison with a control in half sized pot, can be result of higher rooting volume, not presence of competitor (Hess and De Kroon 2007; McNickle and Brown 2014). However, this effect can be filtered out, if a control with plants growing in full sized pot is used, in addition to half size pot treatment (McNickle and Brown 2014). Other possibility that may help to filter out pot size effects is relating the root investment with reproductive effort, or other measure of fitness (as in O'Brien et al. 2005).

Third large class of mechanisms or root navigation in competition, after orientation by nutrient gradients and physical obstacles, is signalisation by chemicals. It is well known, that plant roots excrete many organic substances to soil. These are in sum called root exudates, although purpose of their production can differ dramatically. Some of those substances are primarily designed for non-signalisation purposes, e.g. for modification of soil pH or lubrication of root cap (Wen et al. 2007). Nevertheless, there are many groups of exudates that are produced specifically for root communication with other plants or soil microorganisms (reviewed in Bais et al. 2006). Important is that root exudates can inform plants about the presence of competitor without need of touching roots (Mahall and Callaway 1992) and also independently on above mentioned obstacle recognition mechanisms (Biedrzycki et al. 2010). To separate obstacle recognition mechanisms from exudate communication, two methods were used. One is observing root reaction to medium (sterile one, to avoid other effect as microbial plant-soil feedback), that was previously occupied by other plant (Biedrzycki et al. 2010). Other commonly used method is inhibition of exudate communication by addition of active carbon to soil (Mahall and Callaway 1992; Semchenko et al. 2007b).

Traditionally it was assumed, that root exudates can carry information about species identity of competitor (Mahall and Callaway 1992), but the possibility of kin recognition by exudates was not discussed for a long time (Dudley and File 2007; Novoplansky 2009). Possible reason for leaving out this idea was, that non-peptidic substances can hardly code information about relatedness of individuals. However, recently it was shown that excretion of peptidic strands to soil is quite common (Caffaro et al. 2011). And also there is experimental evidence, that several species really recognise kin individuals (Dudley and File 2007; Murphy and Dudley 2009; Biedrzycki et al. 2010; Lepik et al. 2012). Also as mentioned in previous chapter, kin recognition is common in connected clonal plants. Here possible explanation is that roots of connected plant body also communicate via pulses of electric (Baluska and Mancuso 2009) or chemical potentials (Ortuno et al. 1990; Gruntman and Novoplansky 2004). In case of clonal plants, this would mean, that whole connected plant body would have coordinated pace of pulses, using this for self recognition. Once plant body is disintegrated, those pulses

desynchronise, and even daughter plants of clonal origin start to treat each other as strangers (Gruntman and Novoplansky 2004).

It is important to mention that all above described mechanisms probably do not apply to all of the plant species. Some level of nutrient perception is probably common for most plant species. But for example nutrient optima are likely to be strongly dependent on species ecology, and root navigation in nutritionally extreme conditions should differ between species. Exudate communication seems to be common across many species with different life histories from different habitats (Bais et al. 2006). Detection of obstacles and pot sizes was tested on many species that also do not have much in common (*Pisum sativum*, Falik et al. 2005; *Ambrosia dumosa*, Mahall and Callaway 1992; *Abutilon theoprasti, Setaria faberii*, McConnaughay and Bazzaz 1991; *Vicia faba*, *Zea mays*, *Pisum sativum, Quercus sp.*, Darwin and Darwin 1880). The mechanisms of kin recognition are in detail described on *Arabidopsis thaliana* (Biedrzycki et al. 2010), but there is evidence also for other species diverse in their ecology, like *Cakile edentula* (Dudley and File 2007), *Impatiens pallida* (Murphy and Dudley 2009) or *Trifolium repens* (Lepik et al. 2012).

1.3.   Root competition in space and time

Although there is well developed theory and plethora of experimental evidence for root competition strategies in root mass investment (Weiner 1986, 1990; Müller et al. 2000; Gersani et al. 2001; O'Brien and Brown 2008; McNickle and Brown 2014; see chapter 1.1), spatial patterns of roots in competition are much less explored. However, spatial root distributions in competition might be a key for linking root investment strategies with our knowledge on information perception by plants. Information about spatial distribution of roots in competition, would help us for example to decide whether the reaction we observe is nutrients-driven (as assumed for game-off plants), or whether the plants recognise roots of other species by obstacle detection mechanisms or exudate communication (as predicted for game-on plants). Also, spatial distribution of roots may help us with distinguishing root masses dedicated to transportation and nutrient foraging. In addition, there is also more general reason, why including spatial aspect to theories about belowground competition might be important. In motile organisms, spatially implicit models of competition show that competitive exclusion of weaker competitors can be violated by spatially explicit differentiation of niches (see Gause 1934). This is something that could definitely be tested as well for models of rooting behaviour.

The limited amount of information on spatial patterns in root competition is mostly caused by methodological limitations, because there are only few methods how to observe spatial distributions of roots in common garden experiments. One possibility is to cut soil after

experiment to compartments between competing plants and away from competitor, and weight amount of roots in each compartment (Semchenko et al. 2007b). Modification of this technique is splitting the plants' roots into two parts before experiment, and planting one part of root system to pot shared with competitor and other part to non-shared pot (Gersani et al. 1998). Disadvantage of those methods is small spatial resolution and also the impossibility to measure root branching architecture and related characteristics of root systems. Another option is using visual observation of roots in pots with transparent side (Mahall and Callaway 1992) or rhizotrone tubes (for example Padilla et al. 2013). These methods provide quite good evidence on root architecture in terms of branching and root densities, but on the other hand, they say less about mass allocation of roots to different part of the soil, than the cutting method, because estimating root masses from visual data is always less precise than direct weighting. Similarly to those visual techniques, it is possible to use ones that use different shortwave electromagnetic radiation (x-ray or gamma) and allow observation of 3D root systems instead of their 2D imprints on pot side (Svoboda and Bliss 1974; Mairhofer et al. 2013). It is important to say, that compared to simple weighting of belowground biomass in root investment experiments, all those approaches are much more demanding on time and resources. This is probably the reason why most experimental evidence of spatial rooting patterns in competition comes from experiments using compartmentalisation of rooting space to shared and non-shared parts, which are relatively least demanding.

If we simplify the spatial rooting patterns only on evidence of putting roots away from a competitor or towards it, which can be obtained by the compartmentalisation method, it is obvious that plant can have three strategies (Semchenko et al. 2007b). They can have an intrusive rooting pattern, when they put roots preferably towards the competitor, which may indicate an effort to outcompete him. Also they can have avoidant rooting pattern, i.e. plants stop root proliferation to the soil compartments where they expect competitor presence. Third possibility is an unresponsive pattern, when plants do not react at all to presence of competitor. In intraspecific competition, all three rooting patterns seem to exist in different species. For example *Fragaria vesca* has unresponsive rooting pattern (Semchenko et al. 2007b), *Glechoma hederacea* has avoidance pattern (Semchenko et al. 2007b) and pea has intrusive rooting pattern (Falik et al. 2003; O'Brien et al. 2005). Also, there is evidence for combination of intrusive and avoidance rooting patterns in interspecific competition, where one species avoids contact with the other and the other one yields the contact to oppress its neighbour (Padilla et al. 2013; Semchenko et al. 2007b).

Generally, it is not clear, how intrusive or avoidant rooting patterns are related to game-off or game-on strategies. It is obvious that game-off strategy should not be connected with intrusive pattern. If plant navigates only by nutrient gradients, it has no motivation to grow towards

competitor that lowers nutrient concentration. This is formally shown by spatial rooting model of game-off plant (Novoplansky and Cohen 1997). For game-on plants, situation is more complicated. Spatial model of competition between game-on plants shows, that plants involved in tragedy of commons should increase root density in spatially close soil space shared with the competitor, but maximum reach of their root systems toward competitor should decrease (O'Brien et al. 2007). From the model, it is not clear which of those two processes prevails. If it was the first one, it would lead to intrusive pattern, if the latter one, it would lead to avoidance pattern. The experimental evidence for connection between root allocation and spatial rooting strategy is quite sparse. This is because studies usually focus only on either spatial root patterns or difference between root investment in competitive and non-competitive situations. This results in situation, where studies with data on spatial root distribution do not contain controls grown alone in a pot and vice versa. Experiments that combine both these approaches together are Nord et al. (2011) which works with bean, and Falik et al (2003) and O'Brien et al (2005), which both work with pea. Nord et al. (2011) shows, that bean (variety L88) is game-off and has unresponsive rooting pattern. Falik et al. (2003) show that pea (variety Dunn) is game-on, and has the intrusive rooting pattern. In contrast to the O'Brien et al. (2007) model prediction, even the maximal reach of roots towards competitor increases in this experiment. O'Brien et al. (2005) show as well, that pea (variety Little Marvel) is probably game-on and has intrusive pattern. However, if we compare their results with older experiments performed with the same variety of pea, we find that this variety can produce also avoidance pattern (Gersani et al. 1998). This suggests that connection between game-on strategy and spatial rooting pattern might be dependent on specific experimental setup.

There are also experiments showing how root architectural parameters change in competition (Rubio 2001; Caffaro et al. 2011; Nord et al. 2011; Nan et al. 2013). For example Nan et al. (2013) show that spruce roots in competition decrease number of first order roots and thus rhizosphere surface. Or Rubio (2001) shows, bean roots in competition change branching angles. Problem with root architecture is that each study usually measures different set of parameters, which makes difficult any comparison across studies or connection to theory.

Time is another physical dimension that is implicitly considered in root investment models. And as well as for space, it was shown that its explicit inclusion may improve our understanding to the processes in belowground competition. For example Craine et al. (2005) used simulation model to show that mechanism of competition for nutrients might be based on the pre-emptying of the soil before competitor arrives, rather than on lowering the nutrient concentration in the shared soil space. This would mean that the most effective strategy of belowground competition is fast growth from the beginning, when there is the possibility to pre-empt nutrients to competitor. This strategy was found in the system of *Plantago lanceolata* competing with

Festuca rubra (Padilla et al. 2013). Plants of *P. lanceolata* increased rooting effort in the beginning of competitive interaction, which led to supression of *F. rubra*. This happened although *F. rubra* was shown to have root system more suitable than *P. lanceolata* for later phases of competitive interaction. Interestingly, pre-emption mechanism can serve as an alternative explanation of TOC in root competition. Over-proliferation of roots in competition experiments may be caused by an effort to colonize the pot faster than the competitor, rather than by advantageousness of statically larger root system. Also, supply pre-emption could be a good explanation for intrusive rooting pattern. If plants are motivated to pre-empt the nutrients to competitor, it would useful for them to focus their harvesting efforts in places that are potentially available to both plants, rather to the ones that are too far from the competitor. However, direct evidence connecting root investment strategy, spatial and also temporal patterns exists probably only for game-off plants (in above mentioned Nord et al. 2011). This paper shows that beans in their experiment are game-off, have unresponsive rooting pattern and do not show pre-emption strategy. Broader evidence covering game-on plants is missing very likely again due to technical limitations – most techniques for observing roots are destructive which complicates observation of temporal patterns.

1.4.   Questions

In the previous text I suggested several problems and directions that might be important for better understanding plant strategies in belowground competition. Those problems can be divided in two areas, with division line running on the border between understanding general trends and detailed description of phenomena. And also questions I try to answer in my thesis can be divided in those two categories.

First I focus on strategies in investment to root mass. Here it is possible to take benefit from both developed theory and rich experimental evidence, which allows making and testing predictions across many species. Specifically, I ask:

1. What are the ecological and evolutionary patterns responsible for belowground tragedy of commons in intraspecific competition?
2. Compared to the intraspecific competition, should we expect the same or different patterns for interspecific belowground competition?

Next I focus on connection of root mass investment strategy with reaction to nutrient availability and also spatial and temporal patterns in belowground competition. Here the general approach is not possible, because both theoretical background and experimental evidence are mostly missing. Thus I focus on behaviour of single species – *Agrostis stolonifera*. But even this anecdotal evidence is important in two aspects. First, it is a stone in a general mosaic to be built

in the future. Second, assumptions on nutrient economy and neighbour perception underlying root investment theory are thought to be general. Therefore, even a single piece of evidence can be used to test general theory (Popper 1959 pp 74). My questions are:

3. Is *Agrostis stolonifera* a game-on or game-off strategist?
4. How does the strategy change in response to nutrients availability?
5. Is it possible to observe nutrient pre-emption pattern in root competition of *A. stolonifera*?
6. Does *A. stolonifera* have avoidant, unresponsive or intrusive spatial pattern of rooting when facing intraspecific competition for belowground resources?
7. What is the connection between root investment and spatial rooting pattern in *A. stolonifera*? Is it possible to explain the first by the latter?
8. Is the rooting spatial pattern in *A. stolonifera* governed by nutrient foraging economy or detection of neighbour's roots?

In **chapter 2**, I try to answer **questions 1 and 2**. To do this, I use model based on dove-and-hawk population game (Maynard Smith and Price 1973) to separate the effects of environment and phylogeny on rooting strategy. The model predictions are tested using meta-analysis of evidence of belowground strategy for different species.

In **chapter 3**, I try to answer **questions 3, 4 and 5**. I use experimental design similar to the one proposed by (McNickle and Brown 2014) to explore rooting strategy of *A. stolonifera* in competition. This is performed simultaneously in four different levels of nutrients. Nutrient pre-emption behaviour is tested with temporal data on root growth.

Questions 6, 7 and 8 are examined in **chapter 4**. To explore spatial rooting pattern of *A. stolonifera*, I use rhizosphere shape data from competition experiments. Those are further connected with results of chapter 3. Finally, I perform analysis separating effects of overall root density and nutrient availability on plants' root placement.

2. Tragedy of commons and phylogeny

In this chapter I combine current models of root investment strategy for game-off (McNickle and Brown 2014) and game-on (Gersani et al. 2001) plant. I compare performance of those strategies in population game and show how this performance is dependent on ecological conditions, or on processes necessary for securing plants' fitness. I yield separate predictions for interspecific and intraspecific competition. Predictions for intraspecific competition are further tested by meta-analysis.

2.1.   Model

First I try explore what happens, if hypothetical plants following game-on optimisation model (Gersani et al. 2001) and game-off optimisation model (McNickle and Brown 2014) occur together. If we consider a situation in which game-on and game-off plants co-occur in the same bounded system (henceforth referred to as a "pot"), the optimal rooting strategy (according to Gersani et al. 2001) for the game-on plant should satisfy

$$\frac{d\,P_{\mathrm{on}}}{d\,u_{\mathrm{on}}} = \left(\frac{1}{u_{\mathrm{on}} + u_{\mathrm{off}}} - \frac{u_{on}}{(u_{\mathrm{on}} + u_{\mathrm{off}})^2}\right) * \left(R * (1 - e^{-u_{\mathrm{on}} - u_{\mathrm{off}}})\right) +$$

$$\left(\frac{u_{\mathrm{on}}}{u_{\mathrm{on}} + u_{\mathrm{off}}}\right) * (R * e^{-u_{\mathrm{on}} - u_{\mathrm{off}}}) - c = 0$$

Eqn. 1

The optimal rooting strategy for the game-off plant is (according to McNickle and Brown 2014) described by

$$\frac{d\,P_{\mathrm{off}}}{d\,u_{\mathrm{off}}} = R * e^{-u_{\mathrm{on}} - u_{\mathrm{off}}} - c = 0$$

Eqn. 2

where u_{off} and u_{on} are the root masses of game-off and game-on plants, respectively; P_{off} and P_{on}, the nutrient gains expected by game-off and game-on plants, respectively; R is the amount of nutrients in the pot; and c is the cost per unit root mass.

The biologically relevant solution for this set of equations is

$$\{u_{\mathrm{on}}, u_{\mathrm{off}}\} = \left\{\ln\left(\frac{R}{c}\right), 0\right\}$$

Eqn. 3

This means that when game-on and game-off plants co-occur, the game-on plant would have as many roots, and also the same nutrient gain, as if it were alone in the pot (this gain henceforth referred to as *V*). In contrast, the game-off plant would have no roots in this case, and thus zero nutrient gain.

We also know that co-occurrence of two game-off plants leads to resource partitioning, with each of them obtaining *V/2* (McNickle and Brown 2014). Co-occurrence of two game-on plants also leads to resource partitioning, but the individuals pay some extra cost for root over-proliferation (*M,* representing the total for both plants), so they get *V/2-M/2* (Gersani et al., 2001; O'Brien et al., 2008). Thus, we can analyse the potential for coexistence of game-on and game–off plants in a community, within the framework of the classic hawk-dove game (sensu Maynard Smith and Price 1973; see Table 1), where doves are the game-off plants and hawks are the game-on plants. The population equilibrium of this dove-hawk game can be expressed by the equation

$$ H*0 + D*\frac{V}{2} = H*\left(\frac{V}{2} - \frac{M}{2}\right) + D*V $$

Eqn. 4

where *D* and *H* are the frequencies of doves and hawks, respectively, in the population; the left side refers to the dove strategy average benefit and the right side to the hawk strategy average benefit.

		Strategy type of individual encountered	
		game-on	game-off
Gain received by individual with given strategy type	game-on	V/2-M/2	V
	game-off	0	V/2

Table 1: Nutrient gains of game-off and game-on strategists from encounters with other individuals.

In the case of rooting strategies, net gain in game-on plants co-occurrence should always be positive (see Gersani et al. 2001; O'Brien et al. 2008), with *V* greater than *M*, yielding

$$ 0 < \left(\frac{V}{2} - \frac{M}{2}\right) $$

Eqn. 5

This condition (together with the trivial inequality *V/2<V*) means that the equation for population equilibrium cannot be satisfied with both *D* and *H* positive, suggesting complete dominance by the game-on strategy.

However, in real systems, the fitness of individuals does not simply equal their nutrient gain, so the population equilibrium expected by Equation 4 does not fully apply. We can assume that each competitor has a nutrient uptake-to-fitness translating function that reflects phenomena such as growth rate, aboveground competitive ability and reproductive performance (McNickle and Brown 2014; Semchenko et al. 2010). The relationships these functions (which we refer to as *f* and *g*) generate between nutrients and fitness for each competitor should be increasing and pass through the origin, because zero nutrient uptake is likely to be translated into zero fitness. For coexistence, the density of the community should also play an important role: the sparser the community, the greater would be the frequency of a plant having a small rhizosphere overlap with competitors or none at all. Such a plant gains additional nutrients (denoted as *a*) from unoccupied space. If a community is very sparse, a plant might not have any relevant neighbours at all. We denote the frequency of this growing-alone situation as *A*. Incorporating all of these refinements yields the following, modified version of Equation 4:

$$H * f(0 + a) + D * f\left(\frac{V}{2} + a\right) + A * f(V + a)$$
$$= H * g\left(\frac{V}{2} - \frac{M}{2} + a\right) + D * g(V + a) + A * g(V + a)$$

Eqn. 6

This equation, unlike Equation 4, can be satisfied for positive *D* and *H* if *f* is sufficiently greater than *g*, suggesting the possibility of coexistence of both strategies. It means that species with different rooting strategies can coexist in a community, if the game-off species is able to translate nutrients to fitness more efficiently than game-on species.

In communities that are dense, *a* and *A* are equal to zero, thus allowing transformation of Equation 6 to

$$H * f(0) + D * f\left(\frac{V}{2}\right) = H * g\left(\frac{V}{2} - \frac{M}{2}\right) + D * g(V)$$

Eqn. 7

This equation can be satisfied as well, but the equilibrium state is unstable, because once a game-off species, by random fluctuation, gets below the equilibrium frequency, it is penalised more than in the equilibrium state. This is because the frequency of encounters with the game-on individuals increases, and these encounters result in zero fitness to game-off individuals.

This result suggests that in communities that are dense, coexistence of game-off and game-on species is unlikely.

However, if we switch from coexistence of species with different strategies within a community to coexistence of those strategies within a species, situation is rather different. Within a species (according to biological species definition sensu Mayr 1942 pp 102), we can assume that the translating functions f and g are more or less identical. Even when properties (and thus genes) responsible for translating nutrients to fitness have some intraspecific variation, these would mix within species, and both strategies would have similar probabilities to encounter a given translation function. Thus, the mean expected translation function would be the same for genes carrying either the game-on or game-off strategy. Therefore, the population equilibrium should satisfy the equation

$$H * f(0 + a) + D * f\left(\frac{V}{2} + a\right) + A * f(V + a)$$
$$= H * f\left(\frac{V}{2} - \frac{M}{2} + a\right) + D * f(V + a) + A * f(V + a)$$

Eqn. 8

Because the expressions $A*f(V+a)$ are the same on both sides, it can be transformed to

$$H * f(0 + a) + D * f\left(\frac{V}{2} + a\right) = H * f\left(\frac{V}{2} - \frac{M}{2} + a\right) + D * f(V + a)$$

Eqn. 9

For this equation, analogously to Equation 4, a set of inequalities is valid, as follows:

$$f(0 + a) < f\left(\frac{V}{2} - \frac{M}{2} + a\right)$$

Eqn. 10

and

$$f\left(\frac{V}{2} + a\right) < f(V + a)$$

Eqn. 11

This set of inequalities means that if both H and D are positive (and f is strictly increasing), the population equilibrium of Equation 9 cannot be achieved. So, within a species, game-off and game-on plants cannot coexist in equilibrium, and the dominance by game-on plants is the only stable state. The convergence to the state of game-on plant dominance can, however, be very

slow in some situations, for example, if the cost, *M*, of the encounter of two game-on plants is relatively high, or if the encounter rate within a species (and population density) is low. Nevertheless, once the game-on strategy dominates, it is extremely unlikely that the population will ever leave this state. This implies that once the game-on strategy occurs in a phylogenetic clade, it would be very unlikely to disappear – a prediction that can be tested by comparing evidence of the belowground competition strategy employed by species with the topology of the phylogenetic tree.

2.2. Methods

To select papers to be used as data sources I first performed a Web of Science query in which the topic field consisted of (plant AND root AND competition AND (tragedy of commons OR intraspecific OR kin recognition OR self OR non-self)). The papers thus identified (306 in October 2014) were then examined do to determine whether they satisfied all of the following three criteria for final selection:

First, the experimental design must use pairs or groups of *n* individuals of the same species sharing a rooting space of size *V* as a competition treatment, and individual plants with *V/n* rooting space as a control. Controls must be either separated by a solid divider or grown in individual pots. I did not require a second control grown in a double-sized pot, as proposed by McNickle and Brown (2014), because such a strict criterion would not be met in most cases. However; for a survey covering many species, this relaxation of the criterion would be responsible for only random errors and thus should not artificially generate non-random patterns.

Second, plants in the competition treatment must not be clonally connected and must not be specified as belonging to the same clone or as constituting particularly closely related individuals (e.g., sister or parent-offspring pairs used to test behaviour among related individuals).

Third, the statistical test results for the significance of the difference in either rhizosphere sizes or root/shoot ratios must be available for the comparison of the competition and control group. Experiments where either the rhizosphere investments or root/shoot ratios in competitive and control treatments exhibited a significant difference were considered evidence for game-on behaviour. I used the "significant result" method to analyse the results of experiments instead of performing a meta-analysis based on effect sizes. I did this because effect sizes from the experiments are expected to be highly dependent on the particular experimental setup, especially the distance between competing plants and the number of competitors in competition treatments. This method does have the disadvantage that it does not account for false negative

results; in other words, it can yield significant evidence for the game-on strategy, but the absence of such evidence does not necessarily mean that a species is game-off. This must be considered in the interpretation of results.

I found 14 papers fulfilling these criteria, for a list of the papers used, see table 2. In the cases of species *Pisum sativum*, *Phaseolus vulgaris*, *Oryza sativa* and *Zea mays,* the dataset included information about the rooting strategies of two or three different varieties. All these species are field crops whose different varieties likely behave as independent populations with rare gene exchange, and thus probably fulfil the definition of biological species (sensu Mayr 1942) I therefore decided to treat different varieties from separate papers as independent evolutionary lineages, representing the lowest independent data units in our study. Two papers (Falik et al. 2003; Meier et al. 2013) used the same variety of peas (variety Dunn); because both showed similar results, I treated them as evidence regarding a single lineage.

Using Phylomatic v. 3, I constructed a supertree of all evolutionary lineages used in our survey, and mapped evidence of game-on and game-off behaviour onto this tree. I then tested phylogenetic conservatism of strategy by performing a series of four Mantel tests comparing phylogenetic distances between lineages from an undated tree and distances in strategies, with different strategies defined as having a distance of 1, and shared strategies having a distance of 0. For each of the Mantel tests, some of the data were considered differently, such that particular lineages were or were not merged and particular taxa were considered game-on or game off. These treatments and their rationales are described below.

In one treatment, lineages within species were not merged and the three lineages in our study (*Fragaria vesca, Duchesnea indica* and *Andropogon gerardi*) that exhibited root mass increase when grown with neighbours, but did not show significant evidence for the game-on strategy, were all considered game-off. Because the absence of such significant evidence may have been caused by the low statistical power of the experiments (thereby potentially producing false negatives), I also performed a Mantel test with these three lineages considered game-on (and all infra-specific lineages not merged, as in the previous case).

To account for the possible effects of our decision to treat evolutionary lineages separately, I also performed two Mantel tests on the dataset with infra-specific evolutionary lineages merged into species, in situations in which all lineages within species had the same strategy. For *P. vulgaris,* in which each lineage had a different strategy, I left the lineages separate, enabling a more conservative test than if they were merged. In one of these tests, *F. vesca, D. indica* and *A. gerardi* were considered game-off; in the other, they were considered game-on.

For all Mantel tests, I used the Spearman correlation and ran all tests for 9999 permutations. All computations were performed in R (R Core team 2014), using packages vegan (Oksanen et al. 2013) and ape (Paradis et al. 2004).

lineage	strategy	source
Andropogon gerardii	+	(Markham and Halwas 2011)
Avena sativa		(Semchenko et al. 2007a)
Brassica rapa		(McNickle and Brown 2014)
Duchesnea indica	+	(Littschwager et al. 2009)
Fragaria vesca	+	(Littschwager et al. 2009)
Glycine max	*	(Gersani et al. 2001)
Impatiens pallida		(Murphy and Dudley 2009)
Medicago sativa	*	(Yang et al. 2012)
Oryza sativa Azucena		(Fang et al. 2013)
Oryza sativa Caiapo		(Fang et al. 2013)
Oryza sativa IR64		(Fang et al. 2013)
Phaseolus vulgaris Kenya	*	(Maina et al. 2002)
Phaseolus vulgaris L88		(Nord et al. 2011)
Pisum sativum Alaska	*	(O'Brien et al. 2005)
Pisum sativum Dunn	*	(Falik et al. 2003; Meier et al. 2013)
Zea mays Denghai 3719		(Jiang et al. 2009)
Zea mays Jinhai 5		(Jiang et al. 2009)

Table 2: Evolutionary lineages used in meta-analysis, together with references. * is significant evidence for game-on strategy, + is sub-significant evidence for game-on strategy.

2.3.   Results

The search yielded a total of 14 papers with evidence on belowground strategies for 17 evolutionary lineages in 12 species. I found evidence for the game-on strategy in five lineages (see fig. 1). Twelve lineages did not provide significant evidence for the game-on strategy, but three of those (*F. vesca, D. indica* and *A. gerardi*), exhibit behaviour similar to the game-on strategy, although non-significant. The occurrence of evidence for the game-on strategy was significantly correlated with phylogenetic structure (p=0.0028; r=0.4018). This correlation stays significant even if *F. vesca, D. indica* and *A. gerardi* are considered game-on (p=0.0053; r=0.3763) or evolutionary lineages are merged to species (p=0.0353; r=0.2606). However, if both those modifications are applied simultaneously, the result becomes marginally significant (p=0.0562; r=0.2093).
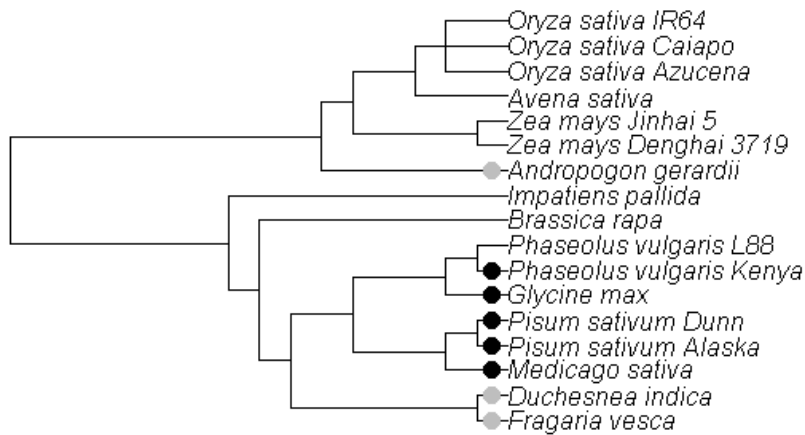

Figure 1: Evidence of game-on strategy across plant phylogeny. Black dots indicate significant evidence for the game-on strategy; grey dots indicate reaction similar to game-on strategy but sub-significant. Occurrence of the game-on strategy is correlated with phylogenetic structure, with p value between 0.0028 and 0.0562 and r between 0.2093 and 0.4018 (Mantel test). Exact values of p and r depend on the method of inclusion of grey lineages and consideration of statistical dependency of lineages within species.

3. Root investment strategy of *Agrostis stolonifera* in competition

In this chapter, I try to explore whether strongly clonal grass species *Agrostis stolonifera* has game-on or game-off root investment strategy in competition. In addition to this, I test whether this strategy changes due to nutrient richness of soil and also how root investment in this species develops in time.

3.1.   Methods

Agrostis stolonifera L. is a temperate grass species with strong clonal reproduction. It exhibits two types of clonal reproduction – intravaginal ramets and long aboveground stolons (Klimešová and Klimeš 2013). The species has wide ecological amplitude ranging from sandy to nutrient rich soils and also it tolerates wide amplitude of moisture conditions, from stands with seasonal drought to temporarily flooded grasslands. Its natural habitats range from grasslands or river banks to ruderal stands (Kik 1990; Kubát et al. 2002). In Czech Republic, 3 cytotypes of this species are widespread – tetraploids, pentaploids and hexaploids (Kubešová 2007). I had several reasons for choosing this species: (i) it is wild living grass, which is a group that seems to be underrepresented in studies on rooting strategy. (ii) It has wide ecological amplitude, so it should possible to widely manipulate conditions without getting out of those that are relevant in nature. (iii) It is clonal species, which eases up experimental manipulation and allows control for relatedness and origin of experimental individuals. (iv) In our workgroup are several theses done on this species recently (e.g. Duchoslavová 2012), so I could benefit from sharing practical experience about behaviour of this species and its cultivation.

For the experiments, six individuals were collected in summer 2013 on localities on southern suburbs of Prague (for GPS coordinates, see appendix 1). The samples were at least 300 m apart from each other, to secure that experimental individuals were not close relatives. The aim of collection was to cover wide scale of possible environmental conditions, where *Agrostis stolonifera* naturally occurs. The biotopes of origin ranged from fallow to shaded bank of fishpond or wet meadow. On each biotope, I wrote down 1 m^2 phytosociological relevé to record original ecological context of individuals (see appendix1). All collected individuals were kept in standardized conditions in growth chamber for at least one month. To avoid maternal effects, only ramets that newly developed in growth chamber were used for experiments. One of individuals (with coding 6) was not used for experiment with root investment, because it did not produce enough material when the experiment was establishing.

During November and December 2015, an experiment testing whether *Agrostis stolonifera* has game-on or game-off strategy was performed. Pairs of ramets (5 – 10 cm of shoot height) of different genetic individuals were cultivated in experimental conditions for six weeks. At the

beginning of the experiment each height to longest leaf and maximal root length of each ramet was measured and the roots were cut to 1 cm length.

There were three treatments to reveal behaviour in competition in this experiment, as proposed by McNickle 2014. In "competition treatment", plant individuals of different genotypes (assembled by numbers 1-2, 2-3, 3-4, 4-5, 5-1) were cultivated in pairs in pots of size 18*15*1 cm always 6 cm from each other. In the "full pot treatment", single plants (again five individuals of different genotypes) were cultivated in flat pots of size 18*15*1 cm, always 6 cm from one side and 12 cm from the other side. In "half pot treatment", single plants were cultivated in flat pots of size 9*15*1 cm, always in the middle.

The whole experiment (competition and two controls) was repeated in four fertilisation levels. Those were 0%, 0.0375%, 0.075% and 0.15% solution of liquid fertiliser Wuxal Super in deionised water. Wuxal Super is a liquid fertiliser with balanced content of nutrients (total N 8%, P_2O_5 8%; K_2O 6%). Recommended concentrations by manufacturer are 0.1-0.4% by volume (Wuxal Super label), however our pilot experiments revealed that in range we used for the experiment, it was possible to observe all the spectrum of nutrient response, from lack of it in 0% to slight over-fertilisation in 0.15% treatment. In 0% fertilisation level, genotype no. 3 was substituted by no. 1, due to insufficient number of ramets in individual 3. Every combination of competition/half/full treatment and fertilisation contained 5 pots, which can be considered independent data points, so whole experiment consisted of 60 pots.

Plants were cultivated in flat pots with one side made of transparent plexiglass. During the experiment, the pot was in skew position with the plexiglass on the lower side. This side was covered by opaque plate to avoid root phototaxis during the experiment. This is a standardized experimental setup used for observing the rooting patterns (Mahall and Callaway 1992). Red sand (stained by insoluble oxides of iron) was used as a substrate in the experiment, because its colour enabled comfortable observation of roots. For competition and full pot treatment, 250 ml of sand was used, and 125 ml was used for half pot treatment. Plants had initially 120 ml (respectively 60 ml in half pot) of nutrients solution and were watered three times during the experiment by 20 ml (respectively 10 ml) of distilled water, which was sufficient to keep substrate moist during all the time. During the experiment all pots were kept in growth chamber Conviron Adaptis A-1000, with day cycle set to 12/12 hours and temperatures to 20/10°C.

After six weeks, the experiment was terminated and the plants were harvested and washed. Whole plants were dried in dryer for 10 hours in 60°C. After this, aboveground and belowground biomass was weighted separately.

In this experiment also data on temporal development of roots were obtained. After 2nd, 4th and 6th week of the experiment, all shoots were counted and measured. Rooting patterns were redrawn on a foil by permanent marker and scanned. The cumulative shoot height and modified pixel count (see next paragraph) in scans served as non destructive proxy of shoot mass and root mass for temporal development analyses. Comparison of shoot and root masses from the end of experiment with those proxies from the end of experiment (6th week) showed tight correlation (R^2=0.885; see appendix 2).

Scans of root drawings with resolution 300 DPI were first aligned to have same position of points where plants were planted using Zoner Studio 12. Those images were further processed in Image J 1.47v (Schneider et al. 2012). First, they were turned to black-and-white bitmaps using colour threshold, filtering out colour of root drawings from the background and other markings on a scan. Those were further cleaned by subsequent "erode" and "dilate" procedures on scale of 3 pixels to get rid of small dust particles. After this, the image was skeletonised, because thickness of line in the drawing did not carry information about root thickness. The skeletonised image was used to obtain pixel counts in each pot, representing root mass (further referred to as "root mass proxy").

In addition, box counting fractal dimension of root system was calculated (using ImageJ) as a measure of root foraging mode. Fractal dimension is commonly used measure to quantify how much organisms focus on harvesting or searching new resources, with higher fractal dimension indicating more of harvesting (Laidre 2003).

All data were processed by linear models. For analyses of fractal dimension, root and shoot masses from the end of experiment, two way ANOVAs with fertiliser concentration and competition treatments as factors were used. To explore contrasts in significant multilevel factors, Tukey HSD tests were applied. I also tried to explore temporal patterns in root allocation from root and shoot biomass proxies from 2nd, 4th and 6th week. To do this I used linear mixed effect model with pot as a block.

Because fertilisation levels 0.0325% and 0.075% (further referred to as "medium fertilisation levels") showed substantially different behaviour from 0% and 0.15% levels (further referred as "extreme fertilisation levels"), all above analyses were run for them also separately, to get better idea about behaviour of plants from non-extreme nutrient conditions. This procedure can be considered multiple testing, so p-values were interpreted using Bonferroni correction with multiplication factor 2 (those p-values are marked by "BC2" note in results).

All data on masses or their proxies in all analyses were log transformed, as the process of plant growth is believed to be rather multiplicative than additive (Blackman 1919, Paine et al. 2012).

The analyses of residual variance justified this decision. Values for both plants in competition treatment were averaged - otherwise it would be necessary to treat them as a block of size two, with unclear correlation structure within a block. All those computations were performed in R 3.12 (R Core team 2014).

Due to technical difficulties, three images of root systems from the 6th week were not available for the analysis. In this case, values of root mass proxies were back-calculated from the root mass – root mass proxy relationship obtained from log-log linear model. Also the values of fractal dimension were supplemented by appropriate fractal dimensions from 4th week. As supplementary data and those supplemented do not take place together in key analyses and their relation was very tight (R^2=0.885; see appendix 2), I believe that this is more reasonable way of work with missing data, than those commonly applied (e.g. supplementation by column mean or group mean).

3.2. Results

The analysis of root masses in *Agrostis stolonifera* shows no evidence for tragedy of commons in root competition in this species. Root mass in competition treatment was not higher than in half pot treatment, but surprisingly it was even significantly lower (fig 2; p=0.036; µ1/µ2=0.62). Also, there was no significant difference between full pot and half pot control (p=0.660; µ1/µ2=0.85). The reaction to nutrients was unimodal. In medium fertiliser levels (0.0375% and 0.075%), plants were significantly larger than in extreme levels (0% and 0.15%; fig 2; p<0.001; µ1/µ2 between 4.00 and 8.31), with no significant differences within those groups. When plants from medium fertiliser levels were analysed separately, the effect of competition treatment became much stronger, showing significant difference between full pot and competition treatment (p=0.006; µ1/µ2=2.05; BC2) and also half pot and competition treatment (p<0.001; µ1/µ2=2.41; BC2) and no differences between full and half pot treatment (p=0.737; µ1/µ2=0.85; BC2). For model summary and contrasts of all levels, see appendix 3.
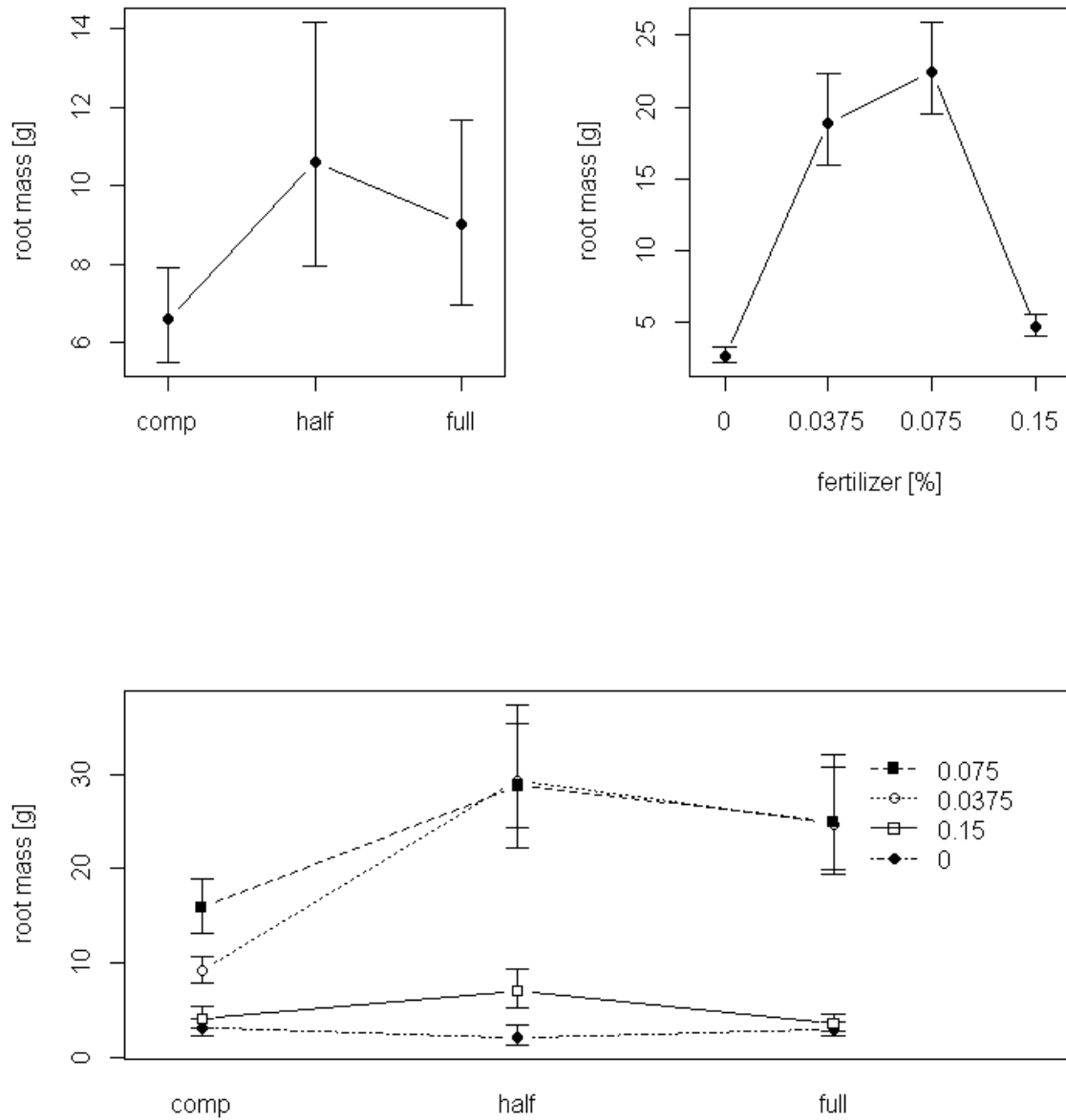
Figure 2: Dependency of root biomass investment on different nutrient levels and competition treatments. Symbols are mean estimates from lognormal distribution, error bars are estimates of ±SE based on lognormal distribution. Upper pane shows main effects of fertilisation and competition treatment, lower pane also their interaction.

Compared to belowground, aboveground biomass did not show any significant difference for half pot, full pot or competition treatment (fig 3, p=0.249). In aboveground, reaction to nutrient availability was unimodal (fig 3), similarly to root mass - nutrient availability relationship. Medium fertilisation treatments were significantly larger than extreme fertilisation treatments (p<0.001; $\mu1/\mu2$ between 2.53 and 13.53). But compared to root masses, aboveground parts of the plants from treatment 0.15% were significantly bigger than those from 0% treatment (p<0.001, $\mu1/\mu2$=3.86), and also appeared more vivid during the experiment (compared to 0% it was growing and creating at least some new stolons). When medium fertiliser levels were analysed separately, the plants in full pot treatment had significantly more aboveground biomass than those from half pot treatment (p=0.001; $\mu1/\mu2$=1.94; BC2) or competition treatment (p=0.011; $\mu1/\mu2$=1.68; BC2). There was no significant difference between half pot and competition treatment (p=0.659; $\mu1/\mu2$=0.86; BC2). For model summary and list of all contrasts, see appendix 4.
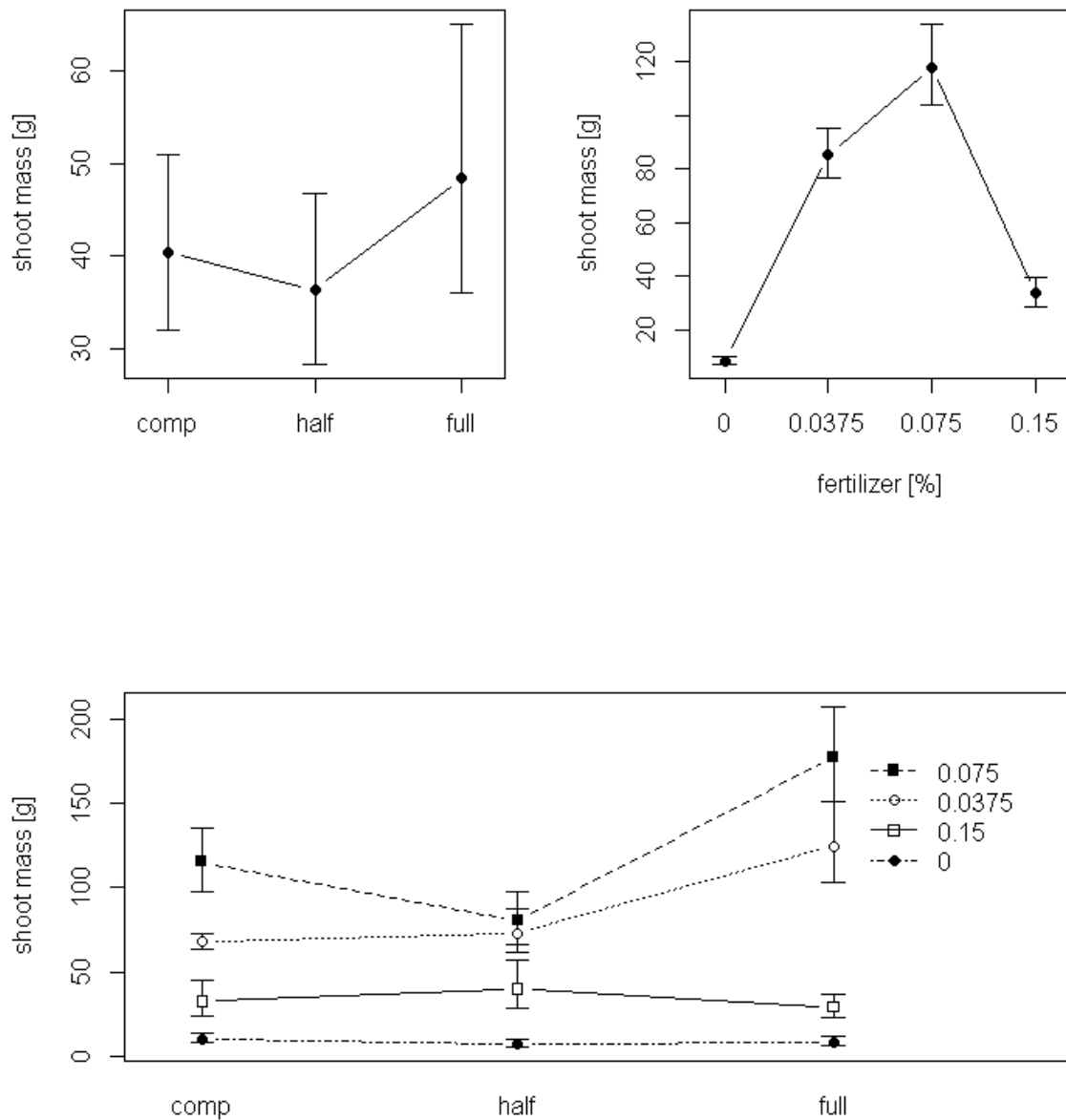
Figure 3: Dependency of shoot biomass investment on different nutrient levels and competition treatments. Symbols are mean estimates from lognormal distribution, error bars are estimates of ±SE based on lognormal distribution. Upper pane shows main effects of fertilisation and competition treatment, lower pane also their interaction.

Plants in half pot treatment had significantly higher fractal dimension of roots than those from competition treatment (fig 4; p=0.008, Δμ=0.05) and sub-significantly higher than those from full pot treatment (p=0.088, Δμ=0.03). Much stronger difference in fractal dimension was observed between different fertilisation levels. Namely, plants with 0% fertiliser had much lower fractal dimension, than plants from other fertilisation levels (fig 4, p<0.001; Δμ between 0.13 and 0.20). It shows that plants grown in the 0% fertilizer focused nearly exclusively on searching more suitable soil patch. Also there was significant interaction term of the model (p=0.019), suggesting that reactions to competition treatments differ in plants in different fertiliser levels. When medium fertiliser levels were analysed separately, the difference between half pot treatment and competition (p<0.001; Δμ=0.10; BC2) or full pot treatment became stronger (p<0.001; Δμ=0.07; BC2).This suggests that in medium nutrient levels, root systems of the plants in the half pot treatment focused more on harvesting than searching for new patches. For model summary and list of all contrasts, see appendix 5.
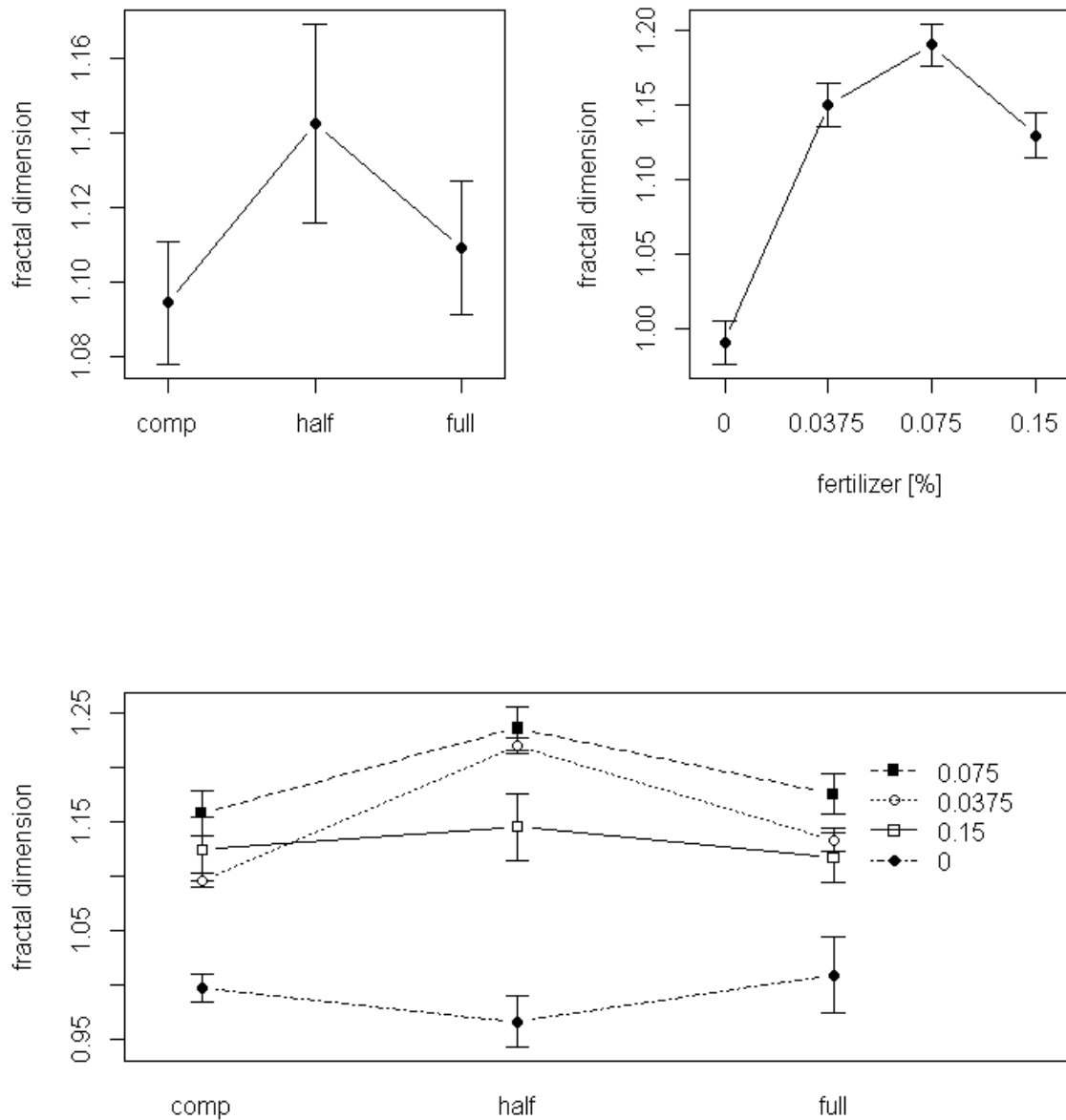
Figure 4: Dependency of root fractal dimension on different nutrient levels and competition treatments. Symbols are mean estimates from normal distribution, error bars are estimates of ±SE based on normal distribution. Upper pane shows main effects of fertilisation and competition treatment, lower pane also their interaction.

Plant roots in all of the treatment combinations significantly grew with the time (fig 5 and 6; $p<0.001$; regression exponent$=0.76$). This growth exhibited significant change with interaction of fertilisation and competition treatment ($p=0.007$; Δ regression exponents up to 0.51). In other words, the growth had different dynamics in different treatment combinations. When plants from medium fertilisation levels were analyzed separately, the interaction with competition treatment became significant ($p=0.001$, Δ regression exponents up to 0.51; BC2), showing that plants in competition treatment grew slower than those from full pot or half pot treatment. For model summary see appendix 6.
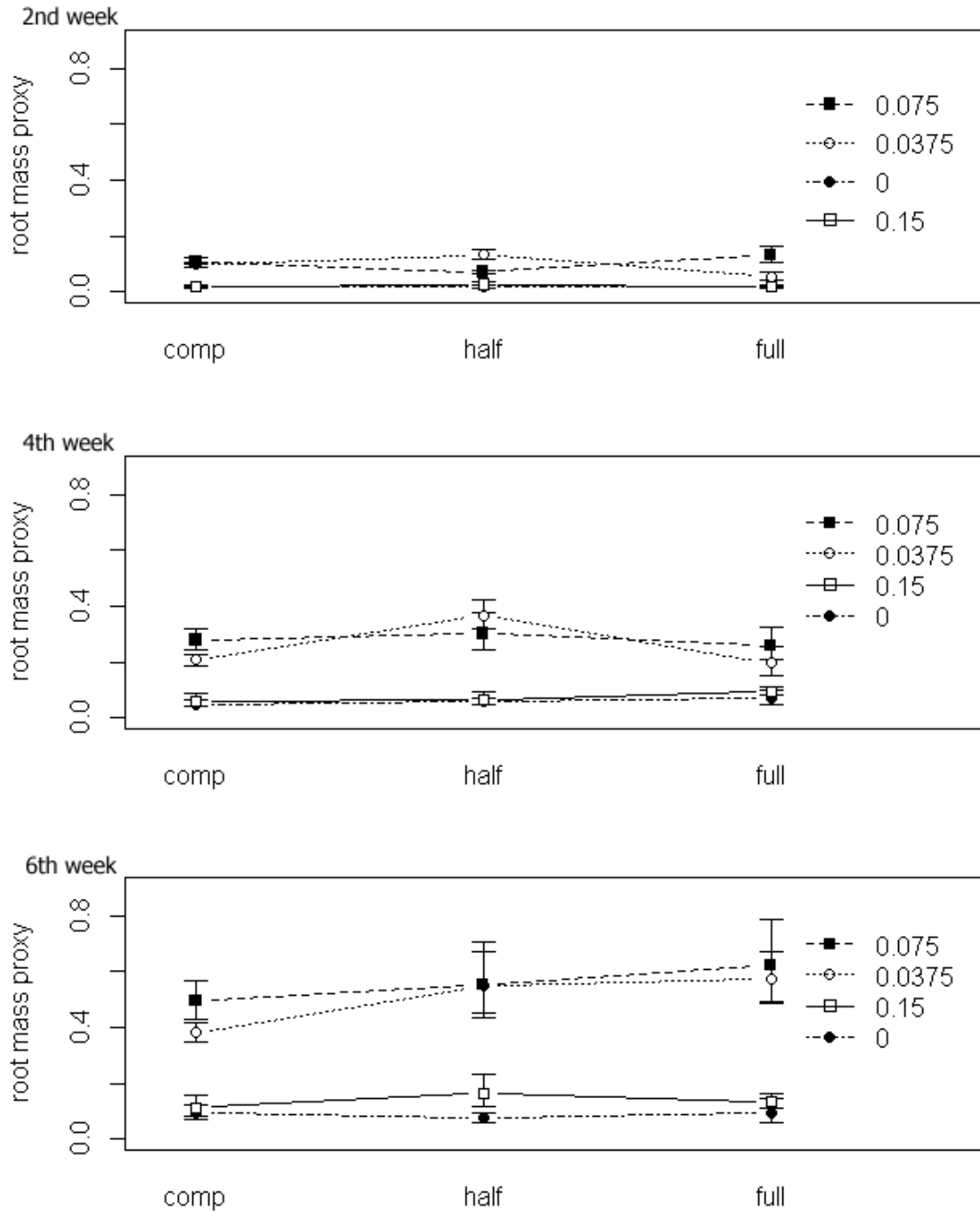
Figure 5: Dependency of plants' root investment on different nutrient levels and competition treatments, and its development in time. Symbols are mean estimates from lognormal distribution, error bars are estimates of ±SE based on lognormal distribution. Upper pane shows situation in 2nd week of experiment, middle pane in 4th week and lower pane in 6th week.
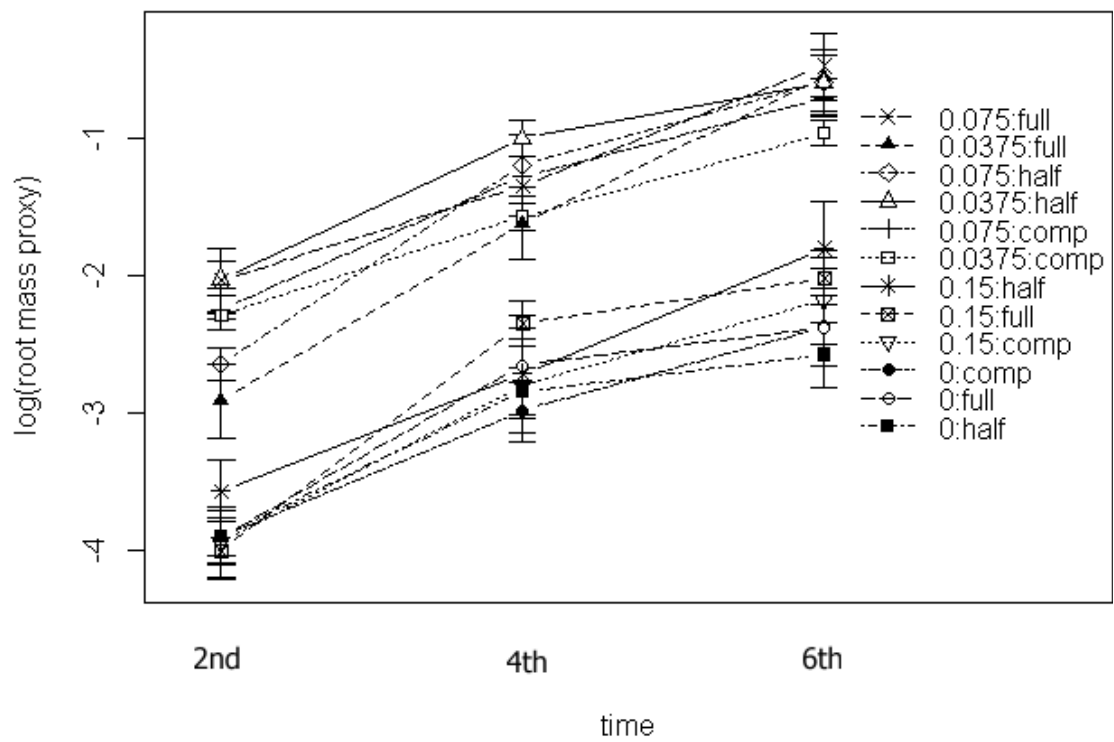
Figure 6: Development of logarithm of root investment in time under different nutrient levels and competition treatments (similar to fig.5, only logarithmic and mixed all together). Symbols are mean estimates from lognormal distribution, error bars are estimates of ±SE based on lognormal distribution. Note that straight line in semi-logarithmic plot denotes exponential growth.

4. Spatial rooting pattern of *Agrostis stolonifera* in competition

In this chapter, I connect results from chapter 3 with spatial patterns of roots in competition of *Agrostis stolonifera*. To do this, I show how allocating roots towards or away from competitor is dependent on fertilisation. Also I test for possible connections between root investment strategy and patterns in space. Finally, I perform analysis revealing whether spatial pattern of roots is dependent rather on nutrient availability or amount of competitors' roots.

4.1.　　Methods

For inference of spatial rooting patterns, the spatial root distribution data from competition treatment in experiment described in chapter 3 were used (I further refer to it as "winter experiment"). In addition, data from two similar experiments performed earlier were added to the analyses:

- First of those experiments was performed in February and March 2014 (I further refer to it as "spring experiment"). It contained only plants in competition treatment in pots of size 18*15*1 cm, but no full size or half size pot controls (which were not absolutely necessary, as the aim was in comparing spatial patterns in various nutrient levels and not biomass allocations). Compared to the experiment in chapter 3, this one did not include nutrient treatment 0.0375%. Also the genotype 6 was used in this experiment along with others, which means, that there were 6 independent data points per treatment, so 18 pots in whole experiment.

- The second experiment was performed in June and July 2014 (I further refer to it as "summer experiment"). It also contained only plants in the competition treatment and only nutrient levels 0.0375% and 0.075%. Genotypes 1-5 were used in this experiment. There were 4 independent data points per treatment, because I did not use combination of genotypes 5 and 1. In whole experiment there were 8 pots.

All other details of those two experiments were identical as for the one described in chapter 3.

In all root scans used it the analysis, points where plants were planted were matched. Each scan was then cut to three parts – part between the two competing plants, left outer part and right outer part (see fig 7). Each of those parts was separately cleaned and skeletonised, according to procedure described in chapter 3. Pixel counts obtained from these skeletonised images were considered a proxy of root biomass put towards or away from the competitor.

To test whether *Agrostis stolonifera* has intrusive or avoidant spatial rooting pattern, centrality measure, counted as

$$log(inner\ root\ mass) - log(outer\ root\ mass) = log\left(\frac{inner\ root\ mass}{outer\ root\ mass}\right)$$

was compared to zero. Positive values of this measure indicated the intrusive pattern and negative values indicated the avoidance. This comparison was done for each nutrient level in each of three experiments separately. Because of unbalanced structure of data with nontrivial correlation structure, Bayesian inference was used instead of frequentist linear models. Measurements were modelled as being drawn from log transformed bivariate normal distributions with mean vector and covariance matrix independent for each fertilisation level from every experiment. Using bivariate normal distribution instead of two independent normal distributions is important, because it is probable that root masses in inner and outer part of one pot are correlated. This correlation can be modelled by covariance matrix of bivariate normal distribution. As priors, I used flat (var=1000; cov=500) normal distribution around zero for means and non-informative (D=2; Vi,i=1000; Vi,j=500) Wishart distribution for covariance matrix. For BUGS code (Spieleghalter 1996) of the model, see appendix 7. The model ran on 10 independent chains for 10000 iterations with 9000 burn-in and convergence was checked visually. All computations were performed in R 3.12 (R Core team 2014), package rjags (Plummer 2014).

Also, I tried to explore connection between root investment strategy and intrusive or avoidant spatial rooting pattern. To do this, I tested whether root mass difference between full pot treatment and competition treatment in experiment from chapter 3 was caused by interactions in the inner part of the pot shared by competing plants or whether outer parts of the root systems are also affected by competitor presence. To do so, I analysed only the outer parts of pots from full pot treatment and competition treatment in same way as in chapter 3. I used "outer" sides of root systems in full pot treatment, i.e. 6 cm wide zone between plant and the pot wall (see fig. 7). These were further cut, cleaned and skeletonised. Their root mass proxy was compared to root mass proxy of outer parts of competition treatment from the chapter 3 experiment. Only pots with 0.0375% of fertiliser were used for the analysis, because for whole root systems, the trend was strongest here. Those data were analysed by log-linear model in R 3:12. Effect sizes and p-values of this "outer" side model were compared with estimates of similar model for whole root systems.
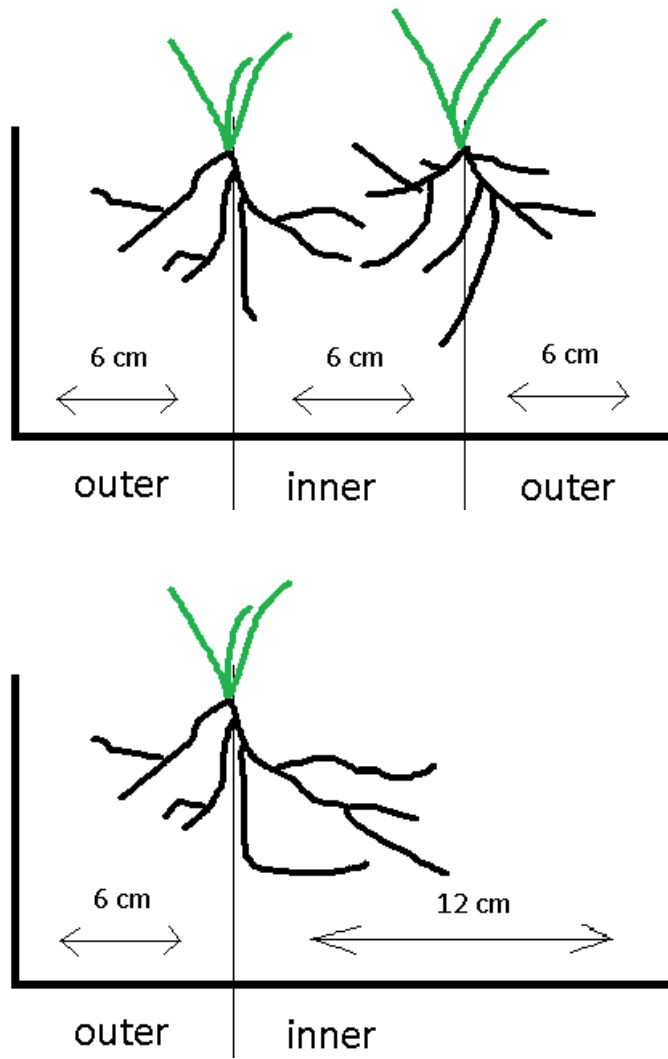
Figure 7: The root scans were divided by positions where plants were planted. This made two "outer" sections and one "inner" for plants in competition treatment (upper pane). For plants in full pot treatments, it divided scans to one "outer" and one "inner" section (lower pane).

To test whether horizontal distribution of root mass is more affected by neighbour presence in rhizosphere (which can be approximated by total root mass in a pot), or presence of nutrients, I performed the following analysis. I took skeletonized root scans in competition treatment from chapter 3 experiment for fertiliser levels 0.0375% and 0.075%. In these fertiliser levels, my previous results suggested that nutrient harvesting was an important process, compared to higher and lower levels, where either searching for resource or over-fertilization artefacts took place. Root skeleton pixels in the skeletonised root scans were summed across raster columns. By this I got vectors of length 1272 in which each value corresponded to amount of root mass in certain horizontal position in the pot (fig. 8). For values in vector fields, three generalized additive models with different predictors were compared: First model included root mass and fertilisation level as covariates, horizontal coordinate of pixel sum as a general spline predictor, and interaction between horizontal coordinate spline and fertilisation level. The second model was similar to the first one, only interaction of horizontal coordinate spline with fertilisation was substituted by interaction with root biomass. The third model contained interactions of horizontal coordinate spline with both fertilisation and root biomass (for model formulas in R formula shape see appendix 8). Those models were fitted in R 3:1 DOPLN, using package mgcv (Wood 2011) and compared using an information criterion (AIC).
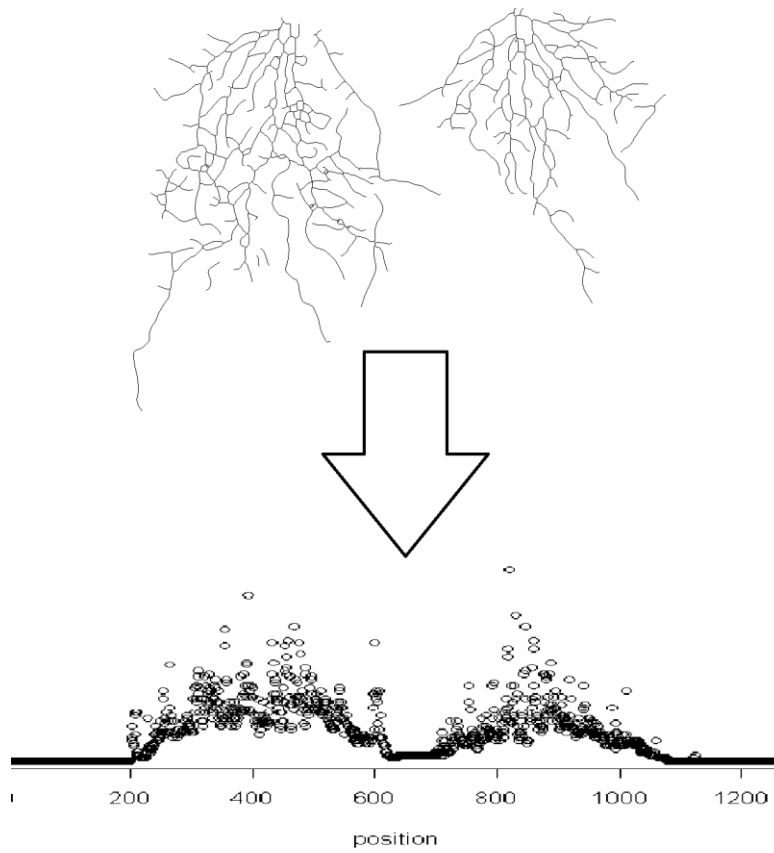
Figure 8: Black pixels in each skeletonised root scan were summed in vertical direction to the vector of length 1272.

4.2.   Results

Plants in most nutrient levels from most experiments exhibited high probability (over 0.975) of having avoidance rooting pattern in competition (see fig. 9). Similarly to results in root investment strategies experiment (chapter 3), this pattern was strongest in medium fertilisation levels (0.035% and 0.075%), and weaker or probably non-existent in extreme nutrient levels (0% and 0.15%). Only combinations of fertiliser levels and experiments that did not exhibit convincing avoidance pattern were thus 0% and 0.15% levels from spring experiment. Here probability of avoidance pattern is under 0.9 for 0% fertiliser level and even under 0.25 for 0.15% fertiliser level.
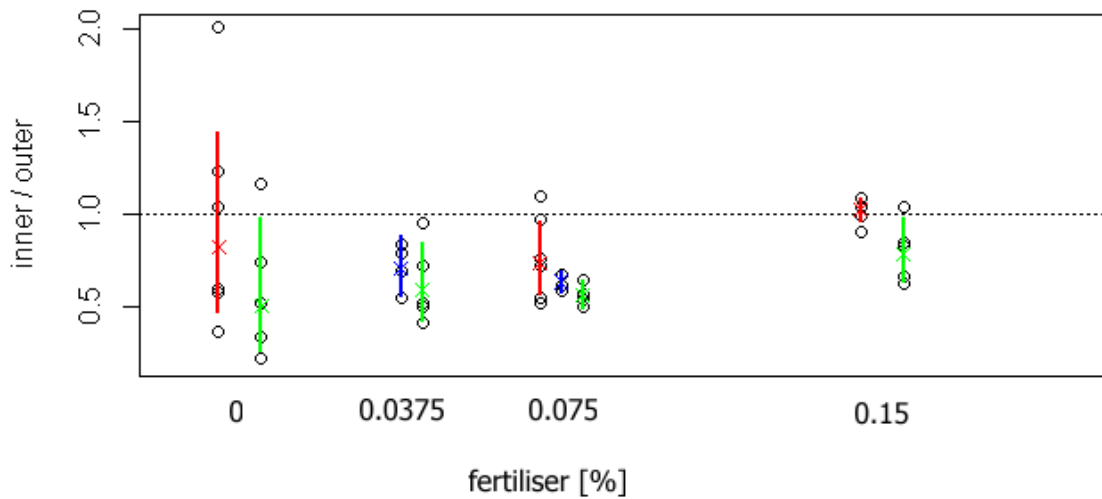
Figure 9: Root investment to inner or outer part of the pot and its dependency on fertiliser. Dots are individual data points. Crosses are medians of posterior distributions for mean ratios from bivariate lognormal distribution. Bars are 95% credibility intervals of those mean fractions. Different colours refer to different experiment runs. Red is spring experiment, blue is summer experiment and green is winter experiment. For sake of better readability, x axis positions of different experiments from same fertiliser level are jittered by value 0.005.

The trends in root masses in competition and full pot treatment did not change and even became stronger, if only root masses from outer part of pots were considered (see fig 10, from $\mu1/\mu2=1.03$ to $\mu1/\mu2=1.17$). However, residual variance in data dramatically increased and caused that the trend became non-significant (from $p=0.020$ to $p=0.276$). In such a situation it is impossible to distinguish, whether outer parts lack signal, or if it is only covered by noise.

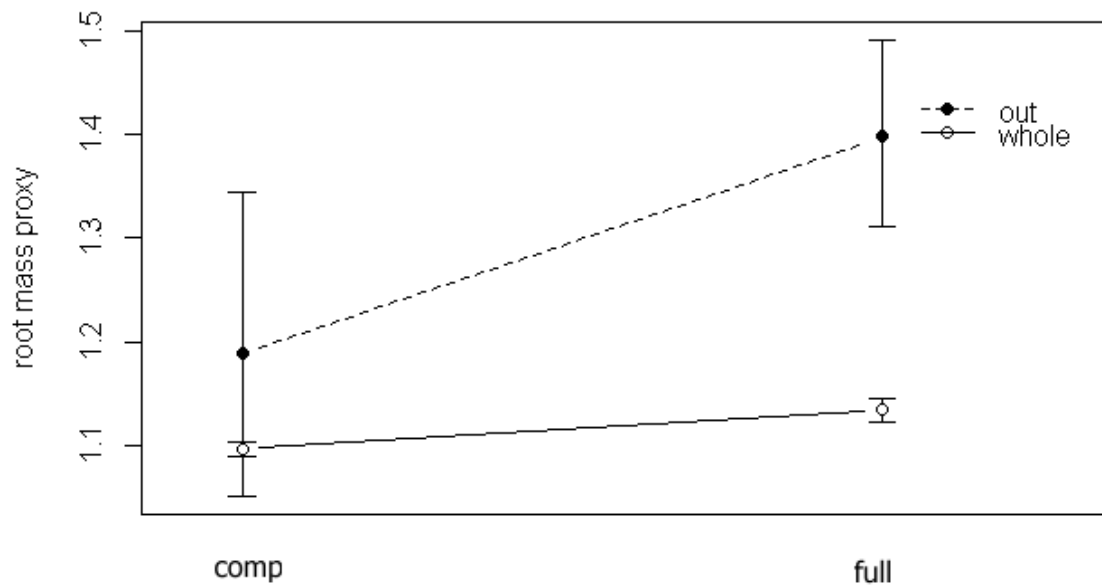**Figure 10:** Root investments in full pot and competition treatment in plants from 0.0375% fertiliser level if whole root systems are considered or only their outer parts. Symbols are mean estimates from lognormal distribution, error bars are estimates of ±SE based on lognormal distribution. All values for outer parts of the pot are multiplied by 3 to get on the same scale as those for whole root systems.

Comparison of GAMs explaining horizontal root distributions by either fertilisation level or root system masses revealed that the one with root masses performs better. This can be explored even visually on heatmaps of horizontal distribution spline interacting with those factors – with root mass the horizontal root distribution spline changes quite dramatically, compared to change with fertilisation (fig 11). However, model with both root masses and fertilisation interactions with spline performed the best (for AICs see table 3). This suggests that spatial root distribution is more affected by neighbour presence, but the nutrient concentrations also play a role.

root shape spline interaction with	AIC
fertiliser level	91595.33
root mass	90182.15
both	88797.44

Table 3: Comparison of AIC for models with shape_spline:fertiliser, or shape_spline:root_mass interactions or both of those.
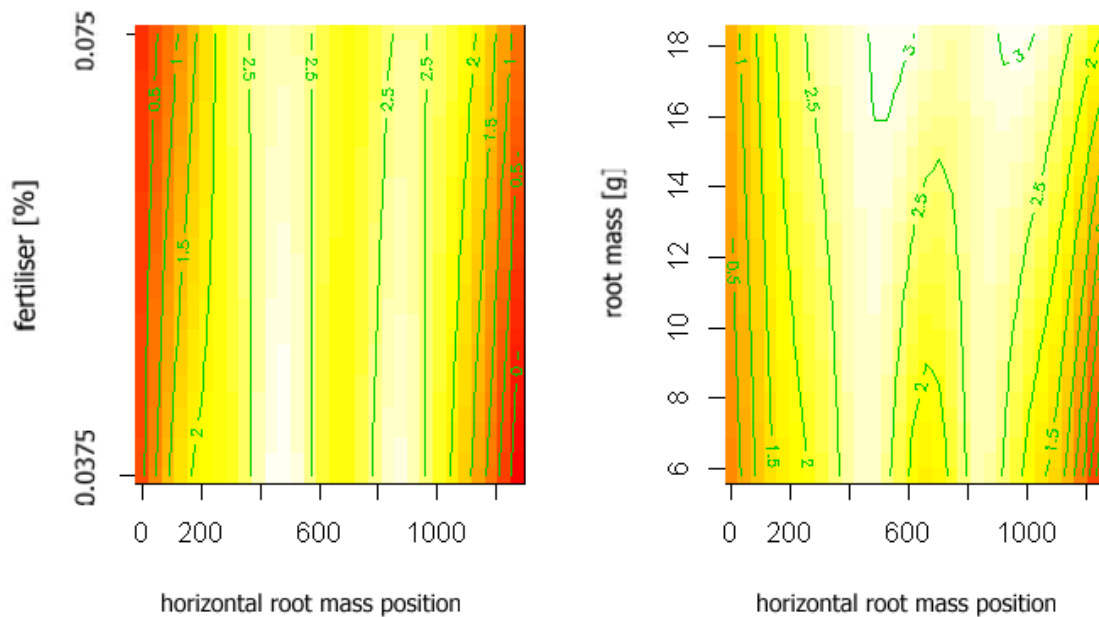

Figure 11: Heat map showing how horizontal distribution of roots changes with amount of fertiliser (left pane) or total root mass (right pane). Warmer colours mean higher values of root mass in certain horizontal position in pot.

5. Discussion

5.1. Tragedy of commons and phylogeny

Using the doves-hawks game modelling framework, I show that the game-on strategy for intraspecific competition should dominate in any ecological conditions where belowground resources are not completely beyond the scope of selection (i.e., nutrients to fitness translation function is strictly increasing). More specifically, the model acknowledges that the strength of selection and thus the probability of adoption of the game-on strategy may be higher in contexts where belowground competition is more important. However, once the game-on strategy dominates in a population, it may not allow appearance of the game-off strategy even if the importance of belowground competition decreases. Extrapolating to a macro-evolutionary scale, this implies that once the game-on strategy occurs in a given evolutionary lineage, it would be very unlikely to disappear even if there was a significant change in the ecology of the species. This contrasts with expectations that the occurrence of the game-on strategy may be determined by the importance of belowground competition in an ecological context (as in McNickle and Brown 2014). Phylogenetic evidence from the data survey supports my prediction. In particular, the most parsimonious interpretation of the phylogenetic pattern I obtained is that the game-on strategy appeared at the base of Fabaceae and disappeared in only one lineage of the six available for this family. This phylogenetic aggregation of the game-on strategy is a sign that the distribution of the game-on strategy has likely been strongly affected by evolutionary relationships.

It is important to mention that there are a few situations in which the model conditions are violated and transition of a game-on population back to game-off might be possible. This backward transition may occur because of group selection. (sensu Wynne-Edwards 1986). The most relevant case of group selection is probably the breeding of crop plants, where usually the groups with the highest yields, not single individuals, are prioritized (Weiner et al. 2010). This might also be the mechanism of the switch to the game-off strategy we documented for *Phaseolus*. However, group selection may also take place in other situations, for example when there are strong facilitation forces between individuals (McIntire and Fajardo 2011). Another situation when the doves-hawks approach may not be valid is when there are strong spatial patterns of reproduction, i.e. daughter individuals are usually placed near mother individuals. This may happen, for example, due to weak dispersal ability or frequent vegetative reproduction. Spatially explicit versions of the dove-hawk game yield different results than the non-spatial version, and if daughter individuals are placed near mothers, it results in worse performance of the hawk strategy (Killingback and Doebeli 1996). It would be interesting to

further focus on these "deviances" from the model, i.e. to map root competition strategies inside phylogenies of field crops, or relate length of clonal spread with root competition strategy.

Apart from support for phylogenetic conservativism, obtained data also suggest that the game-on strategy might be a unique apomorphy for legumes. It is in accordance with the finding that legumes have generally stronger intraspecific competition than other species from temperate grasslands (Semchenko et al. 2013). On the other hand, my result may be affected by the relatively small number of taxa available for the meta-analysis and may change with better phylogenetic sampling or even with a stronger test for the sampled species (especially *Fragaria vesca*, *Duchesnea indica* and *Andropogon gerardi*). In addition, there are also experiments that might show game-on behaviour in other families but could not be included in the meta-analysis, because they did not match the criteria. For example, there is evidence that, in intraspecific competition, *Buchloe dactyloides* preferentially places roots towards unrelated neighbours rather than away from them (Gruntman and Novoplansky 2004). Similar evidence also exists for *Abutilon theoprasti* (Cahill et al. 2010). This phenomenon may be explained by a facilitation process, but the game-on strategy is another legitimate explanation. Also, it has been shown that the game-on strategy may be important for temperate tree seedlings (Haase 2007); however, there were not species-by-species statistics available for these, precluding me from using the relevant paper in our analysis. In general, sampling extent and quality of the meta-analysis enables me to test prediction of phylogenetic clustering, but for mapping game-on strategy across phylogenies, experiment with unified methodology across many species is necessary.

If the phenomenon that we call the game-on strategy is really unique to legumes, explanations of root overproduction that do not use the Tragedy of Commons at all might also be valid. The over-proliferation of roots in the presence of neighbours could for example be explained by root mutualists in legumes, rather than by TOC (this idea is first mentioned in Hess and de Kroon 2007). For example, legumes could make more roots in shared pot due to positive soil interactions via shared populations of root mutualists (as in Rodríguez-Echeverría et al. 2013). However, this explanation seems to be contradicted by the evidence of lower reproductive success of peas and soya in shared pot treatments (Gersani et al. 2001; O'Brien et al. 2005).

For interspecific competition, the story is rather different than for the intraspecific one. Here the model reveals that game-on and game-off strategies can coexist in different species in a community. For this coexistence, species with game-off strategy have to translate yielded nutrients to fitness more efficiently than game-on species. This mechanism of coexistence was already discussed in McNickle and Brown 2014, but was not explicitly linked to interspecific competition. Also there is experimental evidence from mesocosm systems, showing that species with interspecifically game-off rooting behaviour (*Glechoma hederacea*), can indeed coexist

with interspecifically game-on competitor (*Fragaria vesca*), due to better ability of clonal spread (Semchenko et al. 2010). The model also clearly reveals that the coexistence of species with different strategies is possible only in communities, which are not very dense. In other words, the competitively inferior species with better nutrient use efficiency depends on the existence of vacant patches, where it faces only limited competitive pressure from the superior one.

In general I show, that fundamental difference between coexistence of different root investment strategies within species and within community is, that in a community both rooting strategy and ability to translate nutrients to fitness are steadily assigned to each species. This is not a case in intraspecific competition, where individuals of different strategies are crossing and both those properties mix together. Such a result is an interesting indication, that definition of biological species (Mayr 1942), is really a natural concept that makes a deep sense even in fields like community or population ecology.

5.2.   Root investment strategy of *Agrostis stolonifera* in competition

The results clearly show that *Agrostis stolonifera* is not a game-on species. Plants in competition did not invest to root biomass more, than plants in half pot treatment. This further supports my results from the previous chapter. There I found no evidence for game-on strategy in grasses, although sampling for grasses was weak to draw strong conclusions. Also I predicted that game-on strategy should not occur in strongly clonal species, which is a case of *A. stolonifera* as well.

Interestingly enough, *A. stolonifera* shows even more avoidant reaction to competition than is predicted by the ideal free distribution model. For game-off plant it is expected to invest similar amount of resources to roots, regardless of encountering the competitor or being alone in the half sized pot (McNickle and Brown 2014), but *A. stolonifera* invested significantly more to roots if it was grown in half pot treatment compared to root investment in competition treatment. This is behaviour that has never been reported, except of very recent paper on pea (Chen et al. 2015). Important is, that this behaviour probably cannot be explained by simple cost-benefit models, as are TOC and IFD. It suggests that there is no common strategy, or few categories of simple strategies that would describe behaviour of all species. Generally, I see two types of explanation for the observed pattern:

(i) We could assume that root systems in half pot treatment were bigger than expected for the game-off plant. This could be caused for example by reaction to available rooting volume.

Recently, it has been often discussed, that plants can detect available rooting volume and this can affect root mass allocated belowground (McConnaughay and Bazzaz 1991; NeSmith and Duval 1998; O'Brien et al. 2005). This has even been a methodological issue raised against first results showing TOC in root competition (Hess and De Kroon 2007; Laird and Aarssen 2005; Chen et al. 2012; Depuydt 2014). But in contrast to my results, all those papers expect that higher rooting volume should lead to bigger investments to roots. However, it is possible to imagine, that plants in half pot treatment see the possibility to occupy whole pot and secure it against the competitors, whereas plants in competition and full pot treatment consider the pot too large to secure it all. Thus my plants in half pot treatment over-proliferate their root systems, whereas plants from full pot or competition treatment do not. This possibly could be tested in experiment with plants of *A. stolonifera* grown in pots of many different sizes (as done in Chen et al. 2015 on pea). In addition to this explanation, there are plenty of improbable-but-possible methodological explanations of root overproduction in half pot treatment. For example different water regime in less exposed half size pots, or chemicals washing out from the pot stimulating the root growth might play a role. Doubts of this type could be overcome probably only by repeating the same experiment using different materials and probably even different laboratory.

(ii) Other explanation is that plant roots in competition treatment are smaller than expected for game-off plant. This could be caused for example by the fact that plants sharing a pot were not competing, but on the other hand strongly cooperating. This can be really expected in clonal species, which often meet its kin in nature. In case when *A. stolonifera* did not have mechanisms of kin recognition, it might be advantageous to behave gently to all individuals of the same species. To test this possibility, it would be interesting to look for competitive reaction in many species with different degree of clonality and spatial organisation of clonal bodies (i.e. comparing phalanx vs. guerrilla species sensu Doust 1981), as proposed in discussion chapter 5.1. Another cause of smaller root masses could arise from some kind of autotoxicity of competing plants. This is an explanation used in Chen et al. (2015) to explain their results in pea. Argument against autotoxicity is, that although it is commonly found in legumes (Xiao et al. 2006; Liu et al. 2010; Asaduzzaman and Asao 2012), evidence from grasses is quite rare (but see Canals et al. 2005). In addition, severe autotoxicity would probably affect also aboveground biomass of competing plants, which I did not observe. Next possible explanation for root underproduction is, that plants in competition treatment did not recognize that they had intraspecific neighbour, and followed avoidant reaction to expected superior interspecific competitor. Such reaction could easily include economising on actual root system and investing rather to moving to unoccupied patch. Similar reaction was described for *A. stolonifera* competing with *Rumex pallustris* (Mommer et al. 2012), where *A. stolonifera* exhibited to competitor decreased root growth and also avoided nutrient rich patches. However, this

following such avoidant strategy in intraspecific competition would suggests that plants could not distinguish, whether they were facing other avoidant *A. stolonifera*, or superior interspecific competitor.

Patterns in aboveground biomass, compared to belowground, correspond well to patterns expected for game-off plant. Plants from non-extreme fertilisation levels have biggest aboveground biomass in full pot treatment, where they have biggest rooting volume available. In half pot treatment and competition treatment, plants have similar amounts of aboveground biomass. This is quite interesting, because aboveground biomass (which can be considered as proxy of fitness in clonal plant) is dependent on available soil volume, but not that much on how this soil is utilised by roots. I do not see any good explanation for this, except of that variables (root mass, variables extracted from scans) I measured on roots do not carry information on function of roots. However, this is in strong contradiction with plethora of classical literature on relations between root and shoot investment (Weiner 1986, 1990; Gersani et al. 2001; Schenk and Jackson 2002).

Analysis of temporal patterns in root investment revealed, that plants did not exhibit supply preemption behaviour in intraspecific competition. The supply pre-emption is expected to be one of consequences of tragedy of commons (Craine et al. 2005; Craine 2006), so for the species not involved in TOC, this is not a surprising result. Plants in competition treatments exhibited trend, which was even opposite to pre-emption behaviour – they grew roots significantly slower than plants from non-competition treatments. This pattern is an inevitable consequence of overall root suppression in competition discussed in the beginning of this chapter. In other words, the smaller root mass by the end of experiment was approached by slower growth of roots.

All above mentioned patterns in both aboveground and belowground biomass allocation are strongly dependent on fertilisation. All above described reactions to neighbours or pot size are the strongest in medium fertilisation levels. In those fertilization levels, it seems that plant really focuses its efforts on solving issues with competition and rooting space. Compared to this, reaction to competition or rooting volume for plants grown in distilled water or highest fertilisation level is either nonexistent, or has effect sizes under resolution of my experiment. Analysis of fractal dimension provides explanation for this behaviour in case of plants from distilled water. Significantly lower fractal dimension here suggests that plants switched from harvesting of (non present) nutrients, to searching for richer soil patch (similar principle with narwhal foraging in sea in Laidre 2003). In the highest nutrient treatment, the non-existence of competitive reaction can be explained by over-fertilisation. In this treatment, plants had lower biomass also aboveground, which suggests that the concentration of nutrients exceeded the level where nutrients were beneficial and started to be toxic. In this situation, caring about neighbour

also does not make much sense for plant individual. Generally, those results give us strong lesson on choosing right fertilisation level for competition experiments: Over-fertilisation of *A. stolonifera* in levels lower than concentrations recommended by the fertiliser manufacturer (Wuxal Super label) are quite striking for plant that is believed to inhabit mesic environments (Ellenberg 1992; Kubát et al. 2002). It can be partly caused by unnatural properties of liquid fertiliser and this risk of over-fertilisation may be lower with natural or slow release fertilisers. However, it shows that unresponsive patterns in root competition experiments may easily be caused by wrong fertilisation, if they are not performed in either semi-natural conditions or with a species, whose nutrient demands are well known to the experimenter.

5.3. Spatial rooting pattern of *Agrostis stolonifera* in competition

The data show that *A. stolonifera* has avoidant spatial pattern in root competition, i.e. it puts less roots to soil shared with the competitor. This type of response is not very surprising, if we already know that *A. stolonifera* is not a game-on species. Experimental evidence shows that intrusive rooting pattern might be associated with game-on strategy (Falik et al. 2003; O'Brien et al. 2005). And if *A. stolonifera* makes with competitor even fewer roots, than alone (as discussed in chapter 5.2), it is hard to imagine motivation that would force competing plants to put those reduced amounts of roots towards each other.

I tried to test, whether the observed avoidant pattern may be the cause for fewer roots in competition treatment than in full pot treatment, which was observed in chapter 3. To do this, I compared only outer parts of root systems, which are not directly influenced by root contact or nutrient depletion by competitor, with equivalent parts of root systems of plants in full pot treatment. It seems that in outer parts, the trend to make fewer roots in competition has the same direction as in the whole root systems. However, taking only outer parts of root systems dramatically increased variance and this trend became sub-significant. With this amount of data, it is impossible to distinguish whether this result is caused by low statistical power of the test or absence of the signal. If the first was true, it would support interpretation from discussion 5.2, that plants in competition treatment are smaller than expected by IFD strategy. This would lead to interpretations with sophisticated interaction with competitor, as is rooting cooperation, autotoxicity or reaction to expected superior competitor. If there was truly no signal, then the behaviour would match the Novoplansky and Cohen (1998) spatial model of game-off plant. In this model, the plant abandons soil volumes occupied by competitor and does not perform any other reaction in unaffected parts of root system.

As well as in root investment behaviour, availability of nutrients strongly modulates spatial rooting patterns. Strongest avoidance patterns were observed in medium nutrient levels. Explanation for this is the same as for root investment patterns - plants grown in extremely low amounts of nutrients and those over-fertilised focus themselves on different problems than coping with competitor.

A very surprising result is that amount of root biomass is far better predictor of horizontal root distribution than amount of soil nutrients (even after filtering out trivial effect that more root mass simply means higher root pixel counts in general). This contrasts to assumptions of many formal models of root systems (Gersani et al. 2001; Craine et al. 2005; O'Brien and Brown 2008; McNickle and Brown 2014) and also non-formal ideas on root growth in competition (Schenk 2006; Novoplansky 2009). All of those expect, that root growth is tailored to efficient nutrient foraging and thus strongly dependent on amount of nutrients, which can be in further modified by competitor presence. Moreover in game-off plants (McNickle and Brown 2014), the navigation by nutrients should be by definition the only relevant mechanism of root navigation. According to all those models, increasing amount of soil nutrients twice would have dramatic effect on both root investment and horizontal distribution, which is very far from what I observed. The results show, that competing plants' rhizosphere is much stronger shaped by encounters with own or neighbour's roots, than directly by nutrient availability in soil patch. In other words, the observed avoidance pattern is caused rather by regulation of root density towards competitor by competitor presence itself, than by nutrient decrement caused by competitor. This suggests that beside nutrient gradients, *Agrostis stolonifera* uses also other cues in root navigation.

The real mechanism, by which this species regulates its horizontal root placement, can thus be physical contact (as shown by Mahal and Callaway 1992 for *Larrea tridentata*), obstacle recognition mechanism (as shown in Falik et al. 2003 for *Pisum*), or some kind of regulation by root exudates (as shown in Mahal and Callaway 1992 for *Ambrosia dumosa*; in Bierzicky et al. 2010 for *Arabidopsis thaliana*). However, my current results only serve to explore the role of navigation by nutrient gradients. More specific experiments are needed to distinguish whether spatial response to competitor's roots is governed by physical root contacts, physical obstacle recognition mechanisms or exudate communication. For example, the effect of physical root contacts could be tested by observation of time development of spatial rooting patterns with finer time sampling than was done in my experiments. Also, to distinguish effect of exudate communication, it may be valuable to perform an experiment with addition of activated carbon (sensu Mahal and Callaway 1992, Semchenko et al. 2007).

6. Conclusions and future directions

In my thesis I focused on two major issues. First, I tried to determine ecological and evolutionary forces that allow emergence of tragedy of commons in belowground competition of some species, while it does not occur in belowground competition of other species. Second, I tried to describe in detail the rooting strategy of *A. stolonifera* and test the mechanistic assumptions that underlie theoretical models of root investment strategies.

In chapter 2, I show that if the intraspecific Tragedy of Commons really takes a place in nature, we have quite good reasons to think that it will be phylogenetically clustered. There are a few exceptions from this pattern. For example we can expect that field crops or plants that are strongly clonal will not be involved in TOC, even if their ancestors were. If we look at the phylogenetic distribution of TOC, we indeed see clustering of this strategy. Rather problematic is only the fact, that according to our evidence, TOC strategy occurs only in *Fabaceae*. This suggests an alternative explanation: root over-proliferation with neighbours could be explained by aspects of very specific ecology of legumes, and real TOC in root competition does not necessarily exist. Interestingly enough, those findings probably apply only on intraspecific competition, not on interspecific one. This is because in interspecific competition, rooting strategy may strongly interfere with other aspects of plants' life histories, as are aboveground competition, or reproduction.

In chapters 3 and 4, I show that *A. stolonifera* has reactions rather different to what would be assumed by current ideas and models of plant growth in competition. Namely, it has decreased investment to roots in competitive situation. This pattern is associated with avoidant behaviour in space, does not exhibit pre-emption dynamic in time and disappears in over- or under-fertilised plants. Interestingly enough, when plants are neither under or over-fertilised, rooting pattern in competition is governed rather by neighbour presence than by amount of nutrients. There are two possibilities how to incorporate results from *A. stolonifera* in the theory of root investment strategies. First is that we realize, that this species is strongly clonal, and thus an "exception" from the theory, because in nature it is used to play spatial games with neighbours. This can explain under-proliferation of root in competition. But the fact that *A. stolonifera* responds more to neighbour presence than to nutrients can be hardly linked to clonality. Explanation by clonality is also not relevant if the observed deviance from root allocation pattern is caused by over-proliferation in small pots, not by under-proliferation in competition. In this case, we would have to admit that current theory on root investment in competition, dichotomizing plants to game-off and game-on, fails. Or at least its results can be further modified by effects like non-trivial reaction to rooting volume or limited plant ability to estimate the available resource pool.

To summarize my findings, I can say that definitely there are differences between species in root investment strategies in competition. Also it is quite sure that there is a phylogenetic signal in distribution of those strategies. But it is unclear, whether classical explanations of those investment strategies are true. Due to aggregation of evidence for so-called TOC in legumes, it is not clear whether root over-proliferation in game-on species is really sign of more severe competition, or behaviour somewhat connected to nitrogen fixation. Fine scale data on behaviour of *A. stolonifera* suggest that even so-called IFD strategy may in fact lie on different assumptions than it is originally proposed (in McNickle and Brown 2014).In other words, it is not clear whether TOC strategy is a good explanation for root over-proliferation in competition, and also IFD strategy might be inappropriate for some species that do not exhibit root over-proliferation.

However, it is possible to perform tests that would corroborate or reject the idea of TOC and IFD strategies more reliably. One of those could be a big experiment, looking for intraspecific game-on/game-off behaviour in many species simultaneously. In such a study, it would be possible to get rid of issues connected with my meta-analysis approach, as is poor control of false negatives or suboptimal coverage of phylogeny and ecological types of plants. This would allow testing my predictions on phylogenetic distribution of game-on strategy, and thus also indirectly examine the whole idea of tragedy of commons in plant belowground competition.

In addition, it would be possible to focus on taxa, where I predict behavioural deviations from my model. For example in field crops, it would be interesting to map relations between different varieties and their breeding history, and connect this to rooting strategy. This kind of study would be quite interesting even if the whole TOC/IFD concept was eventually rejected. Behaviour of field crops in competition is not only important for plant ecology (Weiner et al. 2010), but also for agricultural applications (for example Hauggaard-Nielsen et al. 2001), and such a comparative phylogenetic study could help with finding new ways for breeding crops. Another group, where interesting behaviour is predicted, are clonal plants. Although there are many studies on competitive behaviour in clonal species, a synthetic study relating degree of clonality to competitive behaviour seems to be missing. Also in this case, such a study would be interesting even outside framework of TOC. Its results could serve for example for better understanding of clonal plants role in communities.

Also it would be extremely useful to perform more studies similar to this on *A. stolonifera* for different species. Especially, it would be useful, if they were done on plant that does not fall to exception-from-model categories, as are field crops and clonal plants. A great disadvantage of my study is, that I chose a plant that could not be fully used to test the model - it is clonal and thus falls to exception from it. (Practical reason of this is, that the choice of species was done

before results of modelling part of my thesis were available.) In order to reconcile the debate on TOC/IFD concept assumptions, it would be interesting to see how more typical game-on and game-off species react to nutrients, rooting volumes and what are their spatial rooting patterns.

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8. Abbreviations

AIC   an information criterion (or Akaike information criterion)

ANOVA   analysis of variance

BC2   tag used for indicating, that Bonferroni correction should apply for interpretation of p-value

GAM   generalised additive model

game-off   plant following the strategy leading to ideal free distribution

game-on   plant following strategy leading to tragedy of commons

IFD   ideal free distribution

TOC   tragedy of commons

Tukey HSD post-hoc test based on honest significant difference method

9. Appendices

Appendix 1 – Identities of experimental individuals

Appendix 2 – Correlation between root mass and root occupancy from scan

Appendix 3 – Root mass contrasts

Appendix 4 – Shoot mass contrasts

Appendix 5 – Fractal dimension contrasts

Appendix 6 – Time model

Appendix 7 – BUGS code

Appendix 8 – GAM specification

Appendix 9 – MCMC summaries